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Report of the Biological Survey of Mutsu Bay.

15. Sipunculoidea.[1]

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(With Pls I-IV and 15 text-figures)

INTRODUCTION

The Sipunculoidea collected by the Biological Survey of Mutsu Bay are represented by nine species belonging to six genera. Of the nine species four may be regarded new to science.

The following is the list of the species.

(1) *Sipunculus nudus* LINNAEUS.
(2) *Siphonosoma mourense*, n. sp.
(3) *Physcosoma japonicum* (GRUBE).
(4) *Physcosoma scolops* (SELENKA et DE MAN).
(5) *Physcosoma glaucum*, n. sp.
(6) *Phascolosoma zenibakense* IKEDA.
(7) *Phascolion ikedai*, n. sp.
(8) *Dendrostoma blandum* SELENKA et DE MAN.
(9) *Dendrostoma hexadactylum*, n. sp.

Here I express my deep gratitude to Professor Dr. S. HÔZAWA for his help rendered me during the course of this present work. I also express my hearty thanks to Professor Dr. S. HATAI, Professor Dr. W. FISCHER, Professor Dr. VAN DER HORST, Professor Dr. L. CUÉNOT, Professor Dr. R. V. CHAMBERLIN, Dr. A. TEN BROEKE, Professor Dr. T. KAWAMURA, Professor Dr. T. KOMAI, Professor Dr. T. UCHIDA, Mr. Y. OZAKI, Mr. M. UÉNO and Mr. S. TAKATSUKI, for their acts of kindness in various ways.

DESCRIPTION OF THE SPECIES.

Key to the genera of Sipunculoidea found in Mutsu Bay.

I. Longitudinal muscle layer separated into bundles.

   A. Hooks absent.

      1. Tentacles leaf-like. Body surface divided into small rectangular areas. Introvert with tall, triangular scale-like papillae posteriorly directed. . . .. Genus *Sipunculus.*

      2. Tentacles filamentous. Body surface shows no rectangular areas. No prominent scale-like papillae on the introvert... .. .. Genus *Siphonosoma.*

   B. Hooks present on the introvert. Filamentous tentacles arranged in a single semicircle above the mouth. Genus *Physcosoma.*

II. Longitudinal muscle layer continuous.

   A. Two segmental organs.

      1. Hooks absent. Numerous tentacles arranged in one or many rows around the mouth. ... . Genus *Phascolosoma.*

      2. Large hooks scattered on the introvert. Tentacles dendritic. . . .. Genus *Dendrostoma.*

   B. One segmental organ. The worms generally inhabit the Molluscan shells. ....Genus *Phascolion.*

Genus SIPUNCULUS LINNAEUS.

Each of the longitudinal and circular muscle layer is separated into bundles. Leaf-like tentacles present around the mouth. Hooks absent. Four retractor muscles present. Intestinal convolution coils spirally around the spindle-muscle terminating free from the body-wall, and is fastened to the body-wall by means of numerous fixing-muscles arising from the latter. Two segmental organs exist. The Polian canals run along the oesophagus. Integumental canal runs along each of the longitudinal muscle-bands. Triangular scale-like papillae are distributed on the introvert.

1. **Sipunculus nudus** LINNAEUS.
(Pl. I, Fig 1, Text-fig 1)

Sipunculus nudus, LINNAEUS, 1766, Syst. Nat. 12th edit p. 1078, W. KEFERSTEIN, 1860, p. 1; 1865, p. 418–419; 1867, p. 44–45; W. BAIRD, 1868, p. 77; J. ANDREAE,

1881, p. 477–481; 1882, p 201–258, Pls. XII–XIII, E Selenka, 1883–1884, p. 92, 1885, p 22, H Ward, 1891, p 143–182, Pls. I–III; A Shipley, 1883, p. 327, 1899 (2), p 158, W Fischer, 1895, p 9, 1914 (2), p 1–28, 1922 (2), p. 5, Pl XXVI, Figs 5–6, S Metalnikoff, 1900, p. 261–322, Pls XVII–XXII; C Sluiter, 1902, p. 5; H Augener, 1903, p 297–371, I. Ikeda, 1904, p 31; 1905, p. 169, R Southern, 1913 (1), p 1–46; J Gerould, 1913, p. 428, R Chamberlin, 1920 (2), p 30, L Cuénot, 1922, p 14, 1927, p 249, A. Ten Broeke, 1925, p 2

Two specimens of this species were obtained by Professor S. Hôzawa at Moura in July, 1926.

They are 140–200 mm in total length, and 10–13 mm. in width. The introvert which is half withdrawn, is about 20–30 mm, long, and is much narrower than the trunk.

The surface of the trunk excluding the introvert is closely beset with small rectangular areas which are formed by the intersection of the longitudinal and circular muscle-bands. The skin of the body is thin and is somewhat translucent. In alcoholic specimens it is nearly white, tinged with slight yellow. At the base of the introvert the skin is slightly brown in colour. The introvert is covered with numerous scale-like papillae which are triangular in surface view with their vertices directed posteriorly. Hooks are absent on both the introvert and the trunk. The tentacular membrane consists of a pair of (right and left) broad lobes, each of which is divided into several leaflets. Of these leaflets, those lying dorsally are larger than the others lying ventrally.

The longitudinal muscle layer is divided into 29–30 separate bands which do not anastomose except in the anterior region of the trunk. In the introvert they fuse together forming a continuous sheet. The circular muscle layer is also divided into numerous bands. Two pairs of short retractor muscles arise at the same level from the inside of the body-wall. The ventral pair (Text-fig. 1, vr) is attached to the 2nd—5th longitudinal muscle-bands, while the dorsal pair (Text-fig. 1, dr) is connected to the 9th-12th of the same. A spindle-muscle (Text-fig. 1, sm) which springs from the 15th longitudinal muscle-band in front of the anus (Text-fig. 1, a), is not fixed posteriorly to the body-wall. Numerous fixing-muscles (Text-fig. 1, fm) which arise from the body-wall and the retractor muscles, are attached to the intestinal convolution (Text-fig. 1, ic). The intestinal convolution (Text-fig, 1. ic) consists of about 20 spirals and its anterior half shows double spirals. The

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Polian canals (Text-fig. 1, pc) pass along the dorsal and the ventral
sides of the oesophagus (Text-fig. 1, o). They are simple and have
not any villus arising from them. The rectal diverticulum (Text-fig.
1, rd) is not a globular sac but is an extraordinarily long tube attaining
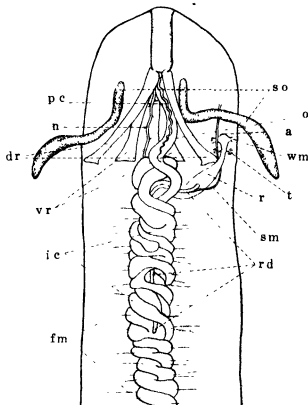
Text-fig. 1 *Sipunculus nudus* LINNAEUS Specimen dissected showing
the anterior part of the body a, anus, dr, dorsal retractor muscles;
fm, fixing-muscles, ic, intestinal convolution, n, ventral nerve-cord, o,
oesophagus; pc, Polian canal, r, rectum; rd, rectal diverticulum, sm,
spindle-muscle; so, segmental organ, t, tuft-like. organ., vr, ventral
retractor muscles; wm, wing-muscles × ⁵⁄₃

the length of about 40 mm. It arises from the rectum (Text-fig. 1, r)
near the point where the intestine begins to coil, and runs posteriorly
along the spindle-muscle ending blindly at the middle of the intestinal

convolution. A pair of tuft-like organs (Text-fig. 1, t) exists near the anus arising from both sides of the rectum and are fastened to the body-wall by means of the mesenteries. The segmental organ (Text-fig. 1, so) consitis of a pair of long tubes of about 25 mm. length and of a yellowish-gray colour. The anterior one-fifth of their length is fixed to the body-wall by means of the mesenteries. Their external openings lie between the 4th and 5th longitudinal muscle-bands at the level far distant from the anus. The ventral nerve-cord (Text-fig. 1, n) does not closely attach to the skin in the anterior region of the body. It is accompanied by two narrow muscles which run along both its sides and which are given off from the first longitudinal muscle-bands at the level of the apertures of the segmental organs. At the level of the introvert-base these two muscles are fused together into a broader muscle which terminates anteriorly in the same point where the nerve-cord also terminates. There is no eye-spot.

Localities. — This species is cosmopolitan and occurs in the Mediterranean Sea, North Sea, Red Sea, Indian Ocean, Adriatic Sea, along the southern coast of the United States of America, Panama, Spain, France, Malaca, the Philippine Islands, Italy, the Bismarck Archipelago, Istria, China, South Australia, etc. In Japan, IKEDA reported this species from Misaki, Tateyama and the Inland Sea. In Mutsu Bay, it was obtained at Moura.

Remarks. — Of the present species I should like to remark on the following points: (1) According to the following authors we learn that the longitudinal muscle layer is separated into 23-34 bands: KEFERSTEIN (1865, p. 419), 30-32; METALNIKOFF (1900, p. 278), 32; IKEDA (1904, p. 32), 30; GEROULD (1913, p. 428), 31-32; CUÉNOT (1922, p. 14), 28-34; TEN BROEKE (1925, p. 2) 30-33. In the specimens from Mutsu Bay, however, they are 29-30 in number. (2) Dealing with a specimen from Key West, GEROULD (1913, p. 428) reported on the segmental organs that nearly half their length is fastened to the body-wall by means of mesentery. In the specimens from Mutsu Bay, it is only the anterior one-fifth of each segmental organ that is fixed to the body-wall, as in the cases reported by other authors.

Genus SIPHONOSOMA SPENGEL.

The longitudinal muscle layer is separated into bands. The circular

muscle layer is usually continuous. Filamentous tentacles encircle the mouth as in the case of the genus *Phascolosoma*. Four retractor muscles are present. The intestinal convolution is fastened to the body-wall at both ends of the trunk. At the anterior part of the intestinal convolution, there occur a small number of fixing-muscles. There are two segmental organs. The Polian canal usually gives off numerous Polian tubules. The integumental canals are cut into many isolated closed sacs, branched very irregularly. No triangular scale-like papillae are present on the introvert.

2   Siphonosoma mourense. n. sp.
(Pl. I, Fig. 2–4)

Many specimens were collected by Professor S. HATAI and Professor S. HÔZAWA at Moura on the 24th of August, 1926, by means of the "*ebiami-dredge*."

In the type specimen (Pl. I, Fig. 2), the body measures about 350 mm. in total length and 10 mm. in thickness. The length of the introvert is almost one-fifth of the trunk.

The surface of the body has a light yellowish-brown colour when preserved in alcohol, and is beset with flat, elliptical papillae measuring about 0.06–0.25 mm. in major axis and 0.04–0.15 mm. in minor axis. They are entirely transparent, and are glandular in structure. Neither hooks nor spines are present on the whole body surface. Numerous finger-shaped tentacles are present around the mouth. They are arranged in twelve regular radial rows, and each row bears about 20 tentacles. The tentacles are arranged in a manner as shown in Pl. I, Fig. 4.

The longitudinal muscle layer is divided into 22 bands. In the anterior region of the trunk they anastomose with each other and they fuse to form a continuous sheet in the region of the introvert. The circular muscle layer is continuous. Of the two pairs of slender retractor muscles, the ventral pair (Pl. I, Fig. 3, vr) is. larger and springs from the 3rd–4th longitudinal muscle-bands at the middle of the trunk, while the dorsal pair (Pl. I, Fig. 3, dr) arises from the 8th–10th longitudinal muscle-bands at the level more anteriorly located. The spindle-muscle (Pl. I, Fig. 3, sm) is fixed to the body-wall at both its ends and gives off two lateral branches at the level where

the intestine begins to coil. Each of these branches is attached to the 8th longitudinal muscle-band at the point about 5 mm. distant anteriorly from the root of the dorsal retractor muscle. The posterior end of the spindle-muscle is divided into several long branches which are fastened separately to the skin at the posterior end of the body. A fixing-muscle (Pl. I, Fig. 3, fm) which springs from the rectum (Pl. I, Fig. 3, r) close to the intestinal convolution (Pl. I, Fig. 3, ic) is attached to the first longitudinal muscle-band on each side by means of its two roots. Broad wing-muscles (Pl. I, Fig. 3, wm) are attached to the rectum near the anus (Pl. I, Fig. 3, a). The crescent-shaped dissepiments are entirely absent on the inner surface of the body-wall. The integumental canals are cut into numerous pieces and form isolated sacs which branch irregularly. Along the longitudinal muscle-bands there occur many KEFERSTEIN's bodies (Pl. I, Fig. 3, kb). They are arranged in several regular longitudinal rows and all lie in front of the apertures of the segmental organs. They are small elongate blind tubules, and are soft to the touch. The long oesophagus (Pl. I, Fig. 3, o) is accompanied by a Polian canal (Pl. I, Fig. 3, pc) from which numerous short Polian tubules arise. The intestinal convolution (Pl. I, Fig. 3, ic) consists of about 30 spirals and these are fixed posteriorly to the body-wall by means of the spindle-muscle. The rectum bears no diverticulum. The anus (Pl. I, Fig. 3, a) is situated between the 10th–11th longitudinal muscle-bands on the dorsal side of the body. Two segmental organs (Pl. I, Fig. 3, so), forming a pair of slender and tolerably long tubes of a reddish-brown colour, measure about 30 mm. in length and are entirely free from the body-wall. Their external apertures lie between the 3rd and 4th longitudinal muscle-bands almost at the same level as the anus. None of the eye-spots can be detected on the ganglion.

    Locality. — Moura.

    Remarks. — This new species seems to be closely allied to *Siphonosoma cumanénse* (KEFERSTEIN), *Siphonosoma edule* (SLUITER), *Siphonosoma billitonense* (SLUITER), *Siphonosoma carolinense* SPENGEL and *Siphonosoma novae-pommeraniae* FISCHER. The main characteristics by which these species may be distinguished from each other are shown in the following table:

   I. Hooks absent.

    II. Apertures of the segmental organs not behind the anus.

      III. Papillae on the introvert not exceedingly tall.

        IV. 20–30 longitudinal muscle-bands (exceptionally 18–21 in *Siphonosoma carolinense* SPENGEL).

          V_1. Crescent-shaped dissepiments present.

                         .*Siph. cumanense* (KEFERSTEIN).

                           Siph. edule (SLUITER).

                 . .   .. *Siph. billitonense* (SLUITER).

                     .. . *Siph. carolinense* SPENGEL.

          V_2. Crescent-shaped dissepiments absent.

             VI_1. Each of the retractor muscles arises from nearly the same level. . *Siph. novae-pommeraniae* FISCHER.

             VI_2. Each of the retractor muscles arises from different levels. . .. *Siph. mourense*, n. sp.

  AUGENER (1903) reported that, (1) the KEFERSTEIN's bodies are found only in *Siphonosoma cumanense* (KEFERSTEIN) ; (2) in the Indian specimens they were mostly present on the anterior region of the body-wall, while in the American specimens they were found both on the anterior and the posterior regions of the body-wall ; (3) they were not always closely attached to the body-wall as mentioned in the description given by KEFERSTEIN ; (4) the bodies are regular or irregular in arrangement. IKEDA (1904, p. 34) reported on these bodies of the Japanese specimens that : these structures, " Ovale Gebilde ", are seen as small oblong bodies closely adhering to the inner surface of the body-wall in *Siphonosoma cumanense* (KEFERSTEIN), while they are not found at all in *Siphonosoma amamiense* (IKEDA). SPENGEL (1912, p. 271–272), however, pointed out that the bodies are the characteristic of the genus *Siphonosoma*. Lately, FISCHER (1926, p. 105) recorded these bodies under the term of " Zottenartige Anhänge " in the case of *Siphonosoma novae-pommeraniae*. In the specimens from Mutsu Bay the KEFERSTEIN's bodies are observed as rather elongate sacs which are arranged in several regular rows.

<div align="center">Genus PHYSCOSOMA SELENKA.</div>

  The longitudinal muscle layer is separated into bands. Finger-shaped tentacles are arranged in a semicircle above the mouth.

Hooks are usually present. There are two or four retractor muscles. A spindle-muscle around which the intestine coils siprally is fixed to the body-wall at both ends. One or more fixing-muscles are present. There are two segmental organs. The dermal papillae are remarkably large. The dorsal vessel is generally simple.

Key to the species of *Physcosoma* found in Mutsu Bay.

I. Hooks strongly curved at apex. Body shows a yellowish-brown colour.

    A. The canal-like space in the hook is simple. No pigmented transverse markings on the dorsal side of the introvert.

                 .   *Physcosoma japonicum* (GRUBE).

    B. The canal-like space in the hook is forked. Several pigmented transverse markings exist on the dorsal side of the introvert.

    . .   *Physcosoma scolops* (SELENKA et DE MAN).

II Hooks not strongly curved at apex. Body shows a greenish-blue colour   .   *Physcosoma glaucum*, n. sp.

3. Physcosoma japonicum (GRUBE).
(Pl I, Fig 5, Text-fig 2)

Phascolosoma japonicum, GRUBE 1877, Vierundfünfzigster Jahresbericht der Schles Gesellschaft für vaterländische Cultur, Breslau, p 73

Phymosoma japonicum (GRUBE), E SELENKA, 1883, p 220-222, 1883-1884, p 76, Pl II, Figs 18-19, Pl X, Figs 145-146, 1885, p 21, W. FISCHER, 1895, p 12; I IKEDA, 1904, p 22

Physcosoma japonicum (GRUBE), A OSTROUMOV, 1909, p 319-321, W. FISCHER, 1914 (2), p 1-28, 1922 (2), p 14, R CHAMBERLIN, 1920 (1), p 5.

Several specimens of this species were taken off Tairadate, off Umayajiri and off Ozawa

The body (Pl. I, Fig 5) has a total length of 30-57 mm., and a thickness of 2-7 mm. The introvert is about one-third of the total body-length, and much narrower than the trunk.

The skin is opaque, yellowish-brown in colour, and convered with numerous large papillae of a deep brown colour. They are more crowded in the region of the introvert-base and the posterior end of the body. These papillae are somewhat elliptical in the surface view, and each is provided with a small aperture in the center. Each papilla is thickly covered with numerous polygona chitinous plates. At the introvert-base and at the posterior end of the body, these papillae are

extremely tall, measuring 0.095–0.19 mm. in height, while in the middle region of the trunk they are small and low measuring only 0.07–0.105 mm. in height. At the anterior end of the introvert, behind the tentacular crown, there are 27–70 rows of hooks. The hooks are about 0.024–0.066 mm. high and are of a deep reddish-brown colour. Each hook (Text-fig. 2) has a strongly curved apical tooth accompanied by a small blunt accessory tooth in the middle of its concave edge. In side view, the hook shows a canal-like transparent streak which bends sharply in the middle region of the hook. A short transverse bar is present at the base of each hook. Close to the posterior end of the bar, there occur a number of minute warts arranged in one row. Between every two rows of the hooks there are small perforated papillae arranged in a circular row, each of these papillae measuring about 0.01 mm. both in height and width. The finger-shaped tentacles, 14–24 in number, present above the mouth, are arranged in a single semicircle.

Text-fig. 2 *Physcosoma japonicum* (GRUBE) Side view of a hook on the introvert ×400.

The longitudinal muscle layer is separated into 22–26 bands. They are anastomosed here and there with each other, and in the region of the introvert they are fused into a continuous sheet. The circular muscle layer is continuous. Of two pairs of retractor muscles, the ventral pair arises from the 3rd–9th longitudinal muscle-bands in the posterior region of the trunk, while the dorsal pair arises far anteriorly from the 6th–8th longitudinal muscle-bands. A spindle-muscle arises from the body-wall in front of the anus and is fixed to the posterior end of the body. A fixing-muscle springs from the left side of the nerve-cord in close approximation to it, and terminates in the first whorl of the intestinal convolution. A pair of stout wing-muscles is attached to both sides of the rectum. The intestinal convolution has 8–14 spirals. No Polian tubules are found on the Polian canal which runs along the dorsal side of the oesophagus. The rectal diverticulum is absent. The anus is situated almost at the same level with the external apertures of the segmental organs. The segmental organs are brown in colour, and the anterior half of their length is connected

with the body-wall by means of mesentery. A pair of eye-spots are visilbe on the ganglion. There is a pair of gonads along the base of the ventral retractor muscles.

Localities. — Port Jackson; Port Natal; De Castries Bay; Sidney; New Britania and British Columbia. In Japan this species was obtained from Hokkaido, Misaki, Enoura, Enoshima, Satsura and Nokabuta. In Mutsu Bay it occurs at Tairadate, Umayajiri and Ozawa.

Remarks. — The hooks are exceedingly small in size comparing with those of the specimens collected from the other localities. It is reported by SELENKA (1883-1884, p. 76) in his monograph that the tentacles are 28 in his specimen, while we found 14-24 tentacles in the specimens from Mutsu Bay. SELENKA (1883-1884, p. 76) also reported that the fixing-muscle of the species is simple and not forked as in the case of *Physcosoma scolops* (SELENKA et DE MAN), while in the specimens from Mutsu Bay there is not a simple fixing-muscle but a forked one.

4. Physcosoma scolops (SELENKA et DE MAN).
(Pl II, Figs 9-10, Text-fig 3)

Phymosoma scolops, SELENKA et DE MAN, 1883-1884, p 75, Pl II, Fig 17. Pl. X, Figs 138-144, 1885, p 21, C. SLUITER, 1890, p 119, W FISCHER, 1892, p 86, A. COLLIN, 1901, p 304, H AUGENER, 1903, p 297-371, I IKEDA, 1904, p 20, M. A HÉRUBEL, 1904, p 476-480, Text-fig. 1, 1907, p. 221
Physcosoma scolops (SELENKA et DE MAN), A. SHIPLEY, 1898, p 56, 1899 (1), p. 470; 1899 (2), p. 156, 1899 (3), p 1899, 1902, p 135; 1903, p. 174, C. SLUITER, 1898, p 443, 1902, p. 12, W F LANCHESTER, 1905 (1), p. 28; 1905 (2), p 30; 1905 (3), p 36, W FISCHER, 1913, p 98; 1914 (1), p 59-84, Pl. XI, Figs 6-8; 1914 (2), p 1-28; 1922 (2), p 15, 1926 (1), p. 108; I. IKEDA, 1924, p 31, A. TEN BROEKE, 1925, p 6

Many specimens were collected from Futagojima, Asamushi, Tsuchiya, Sai, off Kanita and off Tairadate.

The whole body measures about 25-50 mm. in length and 1.5-4 mm. in thickness in a fully extended state. The introvert is about one-third of the entire body length, and is much narrower than the trunk.

The yellowish-brown coloured body-wall is thin and is covered with numerous large papillae of a · deep brown colour. Each papilla is cylindrical in form with a conically pointed tip and is thickly beset with numerous polygonal chitinous plates leaving a small aperture in

the centre. These papillae found at the introvert-base and at the posterior end of the body, are extremely tall. The measurements of the height and the width of the papillae are shown in the following table:

	Specimen A		Specimen B		Specimen C	
	Height	Width	Height	Width	Height	Width
Papilla from introvert			0.11 mm	0.08 mm	0.08 mm	0 13 mm
Papilla from introvert-base	0.25 mm	0 23 mm	0.35 mm	0,22 mm	0.52 mm	0,17 mm
Papilla from the middle region of trunk	0.12 mm	0,16 mm	0 14 mm	0 15 mm	0.15 mm	0.17 mm
Papilla from the posterior region of trunk	0 29 mm	0 28 mm	0,25 mm	0,31 mm	0.23 mm.	0,27 mm

On the dorsal surface of the introvert there are usually numerous pigmented bands of a dark brown colour Hooks are arranged in 15-25 circular rows at the anterior end of the introvert. Each hook is about 0.036-0.07 mm. both in height and width, and is deep reddish-browm in colour. It has a strongly curved apical tooth and a very small accessory tooth. In a side view of the hook, we notice a canal-like transparent streak as shown in text-fig. 3. A short transverse bar, having several minute warts close to its posterior end, is present at the base of the hook. Between every two rows of the hooks there exist small per-forated papillae about 0.01 mm. in height and width arranged in circular rows. The tentacles are 16-24 in number and are arranged in a semicircle above the mouth.
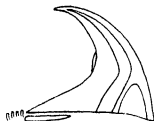
Text-fig. 3   *Phyreosoma sco-lops* (SELENKA et De MAN) Side view of a hook on the introvert ×400.

The longitudinal muscle layer of the body-wall is divided into 20-29 bands. They are usually separated from each other but are often anastomosed. In the region of the introvert they are perfectly fused into a continuous sheet. The circular muscle layer is continuous.

Two pairs of slender retractor muscles are present. The ventral pair (Pl. II. Fig. 10, vr) of the muscles arises from the 2nd–7th longitudinal muscle-bands at the level of one-fourth of the body-length from the posterior end of the body. The dorsal pair (Pl. II, Fig. 10, dr) is narrower than the ventral, and arises from the 5th–9th longitudinal muscle-bands at a level placed more anteriorly than that of the latter. A spindle-muscle (Pl. II, Fig. 10, sm) arises from the body-wall in front of the anus, and is fixed to the posterior end of the trunk. A fixing-muscle (Pl. II, Fig. 10, fm) arises from the body-wall at the left side of the nerve-cord (Pl. II, Fig. 10, n) in close approximation and insert to the first whorl of the intestinal coil. Wing-muscles (Pl. II, Fig. 10, wm) are attached to the rectum (Pl. II, Fig. 10, r) near the anus. The intestinal convolution (Pl. II, Fig. 10, ic) consists of about 5–15 spirals which coil around the spindle-muscle. A simple Polian canal is found only on the dorsal side of the oesophagus (Pl. II, Fig. 10, o). There is no diverticulum upon the rectum. The anus (Pl. II, Fig. 10, a) is situated almost at the same level with the external apertures of the segmental organs (Pl. II, Fig. 10, so). The segmental organs are long tubes of a reddish-brown colour, and their anterior half is fixed to the body-wall by means of the mesenteries. Two eye-spots (Pl. II, Fig. 10, es) are found on the ganglion.

Localities. — This cosmopolitan species has been obtained from the Philippine Islands, Singapore, the Red Sea, Batavia, Zanzibar, West Africa, Funafuti, the Loyalty Islands, the Indian Ocean, Germany, the Maldive and Laccadive Islands, New Zealand, Tasmania, Ceylon, France, British East Africa, Penang of the Malay Peninsula, the Gulf of Suez, Java, Port Natal and New-Britain. In Japan, according to IKEDA (1904, p. 21 ; 1924, p. 31) it was previously collected from the Hokkaidô in the north and from the Riukiu Islands in the south. In Mutsu Bay the species was found at Futagojima, Tsuchiya, Sai, Asamushi, off Kanita and off Tairadate.

Remarks. — Three varieties of this species have been reported by E. SELENKA, M. A. HERUBEL and W. FISCHER : They are *Physcosoma scolops* (SELENKA et DE MAN) var. *mossambicense* SELENKA et DE MAN (1883–1884, p. 76, Pl. X, Fig. 144), *Physcosoma scolops* (SELENKA et DE MAN) var. *adenticulatum* HERUBEL (1904, p. 476–480) and *Physcosoma scolops* (SELENKA et DE MAN) var. *tasmaniense* FISCHER

(1914 [2] p. 1-28). The specimens before me differ from the second
variety in posessing the warts at the base of the hooks, and from
the third variety in the shape of chitinous plates covering the papillae
as well as in the absence of the sharp accessory tooth on the hook.
The first variety, *Physcosoma scolops* (SELENKA et DE MAN) var.
mossambicense SELENKA et DE MAN, according to SELENKA, differs
from the present species in the colouration of the body, in the opaque
skin, in the number of rows of hooks, in the shape of the transparent
parts of the hooks and in the absence of the accessory tooth. In the
specimens from Mutsu Bay, however, those characters above mentioned
seem not to be constant but are rather variable, and thus I am inclined
to consider that *Physcosoma scolops* (SELENKA et DE MAN) and
Physcosoma scolops (SELENKA et DE MAN) var. *mossambicense* SELENKA
et DE MAN are such closely allied forms that it seems to be rather
preferable not to separate them. In several specimens collected from
Tsuchiya and Futagojima, the characteristic pigmented marking on
the dorsal side of the introvert is absent. Refering to this marking,
IKEDA (1904, p. 22) also states : — "The specimens I collected on the
shore of the Aomori Bay, are entirely devoid of the characteristic
markings on the introvert". By SELENKA (1883-1884, p. 75) it is
reported that the number of rows of hooks is 15-17, but in the
specimens from Mutsu Bay they are 15-25. The shape of the trans-
parent canal-like streak of the hook is rather variable and does not
show any constant feature as represented in the figures of SELENKA's
monograph. The accessory tooth is distinctly visible in some of our
specimens, while the others are entirely devoid of it as in the case
of FISCHER's specimens from WAHRBERG (1922 [2], p. 15). According
to SELENKA (1883-1884, p. 75), the height of the hook is 0.07 mm.
while in the specimens from Mutsu Bay it is 0.036-0.07 mm. By
SELENKA (1883-1884, p. 75) and the other authors it is reported that
the number of the tentacles is 12, while in the specimens from Mutsu
Bay it is 16-24. According to SELENKA (1883-1884, p. 75), FISCHER
(1926 [1], p. 108) and LANCHESTER (1905 [1], p. 28), the number of
the longitudinal muscle-bands is 20-21, 22 and 17-19 respectively,
while in the specimens from Mutsu Bay it is 20-29. SELENKA (1883-
1884, p. 75) and IKEDA (1904, p. 20) reported that the fixing-muscle
of this species is forked at the point of attachment to the intestinal

convolution, while in the specimens from Mutsu Bay the fixing-muscle is not always forked, the majority being simple. In one of the specimens from Mutsu Bay there is a globular body attached to the dorsal side of the oesophagus. The body seems to be quite similar to the body reported by IKEDA (1904, p. 23) in the case of *Physcosoma japonicum* (GRUBE).

5. Physcosoma glaucum, n. sp.
(Pl. 1, Fig 6, Pl II, Figs. 7–8, Text-fig. 4)

Two specimens were collected at Urata on the 13th of July, 1926, by Mr. S. TAKATSUKI.

The type specimen (Pl. II, Fig. 7) is small and is about 30 mm. long and 5 mm. wide. The introvert measures about 8 mm. in an almost contracted state.

Both on the anterior and the posterior regions of the trunk the skin shows a light greenish-blue colour, and in the middle it has a greenish tint. Near the anus the skin shows a deeper brown colour. The skin is thin and more or less translucent. The whole surface of the body is covered with numerous papillae which are of a deep brown colour. Those placed in the region of the introvert-base are the largest and those on the introvert are the smallest, as the following table shows.

	Height	Width
Papillae on the introvert	0.06–0.08 mm	0.065–0.11 mm
Papillae at the introvert-base	0.12–0.21 mm.	0.14–0.21 mm.
Papillae in the middle region of body	0.05–0.085 mm.	0.065–0.13 mm.
Papillae in the posterior region of body	0.10–0.185 mm.	0.12–0.18 mm.

About 50 rows of hooks are present behind the tentacles on the introvert. Each hook (Text-fig. 4) is small in size, measuring about 0.04 mm. in height and 0.056 mm. in breadth and is deep brown in colour. The apical tooth of the hook is not so strongly curved as in the case of *Physcosoma japonicum* (GRUBE). A transparent canal-like streak which runs inside of the hook, is sharply bent at the middle.

A short transverse bar and several minute warts arranged in one row are found at the base of the hook. The perforated papillae are scattered sparsely among the hooks. They are about 0 01–0.02 mm. in the diameter of the base. Nine filamentous tentacles are arranged in a semicircle above the mouth.

The longitudinal muscle layer is divided into many separate bands, several of which are anastomosed. They are 28 in number at the middle of the body, while they are 24 at the posterior region. In the region

Text-fig 4 *Physcosoma glaucum*, n sp Side view of a hook on the introvert ×400

of the introvert these bands are entirely fused into a continuous sheet. The circular muscle layer is continuous. Of the four retractor muscles, the ventral pair (Pl. II, Fig. 8, vr) is larger than the dorsal and springs from the 2nd–8th longitudinal muscle-bands at the middle region of the trunk, while the dorsal pair (Pl. II, Fig. 8, dr) arises far anteriorly from the 4th–5th longitudinal muscle-bands. A simple stout spindle-muscle (Pl. II, Fig. 8, sm) which arises in front of the anus is fixed to the posterior end of the body at its extremity. One fixing-muscle (Pl. II, Fig. 8, fm) arises from the first longitudinal muscle-band on the left side of the trunk, and each of its two roots is attached respectively to the oesophagus (Pl. II, Fig. 8, o) and rectum (Pl. II, Fig. 8, r) at the region where the intestine begins to coil into a spinal. A pair of wing-muscles (Pl. II, Fig. 8, wm) are attached to the lateral sides of the rectum near the anus. The intestinal convolution (Pl. II, Fig. 8, ic) consists of 13–14 spirals and its posterior end is fixed to the trunk by means of the spindle-muscle. No diverticulum is present upon the rectum. The anus (Pl. II, Fig. 8, a) is situated slightly behind the external apertures of the segmental organs A Polian canal (Pl. II, Fig. 8, pc), without tubules upon it, is found running along the dorsal side of the oesophagus. The segmental organ (Pl. II, Fig. 8, so) consists of two large sacs of a yellowish-gray colour and each is furnished with a protuberance in the middle. Their external apertures lie between the 2nd and 3rd longitudinal muscle-bands. The anterior one-third of each segmental organ is fixed to the body-wall by mesentery. A pair of eye-spots (Pl. II, Fig. 8, es) is seen on the ganglion. On the internal surface

of the body-wall, behind the ganglion, there is a narrow transverse marking of a deep green colour.

Locality. — Urata.

Remarks. — This new species may be distinguished from the other members of the genus by the shape of the hooks and by the characteristic greenish-blue colour of the skin.

Genus PHASCOLOSOMA F. S. LEUKART.

The longitudinal muscle layer is continuous. Tentacles are finger-shaped or leaf-shaped, and encircle the mouth in one or many rows or groups. Hooks may or may not be present on the introvert. Two or four retractor muscles are present. Generally a spindle-muscle is also present. The anterior portion of the intestinal convolution is fixed to the body-wall by one or more fixing-muscles, while its posterior end is usually free from the body-wall. There are two segmental organs. The dermal papillae are small in most species.

6. Phascolosoma zenibakense IKEDA.
(Pl. III, Figs. 11-12, Text-fig. 5)

Phascolosoma zenibakense, IKEDA, 1924, p. 29, Pl. 1, Fig. 1

Only one specimen was collected by Professor S. Hôzawa and Mr. S. TAKATSUKI on the 23rd of July, 1926, off the Asamushi Marine Biological Station by means of a dredge. The specimen was so well preserved in alcohol that the introvert entirely protruded from the trunk.

The animal (Pl. III, Fig. 11) is tolerably large. The whole body measures about 100 mm. in length and 6-14 mm. in thickness, while the introvert is about 40 mm. long and has a uniform width of about 3.5 mm. The posterior end of the trunk is more or less conically pointed.

The ground colour of the skin is a light yellowish-gray, but at the anal region it shows a deep brown colour. The skin appears nearly smooth to the naked eye, but there can be detected sparsely dispersed small papillae when observed under high magnification. The papillae found on the trunk, excepting the posterior region, are cylindrical in form and are of nearly equal height. Those on the posterior

region of the trunk are inferior in height to those above mentioned. The width of the papillae is variable in accordance with the region of the body where they occur, as shown in the following table.

	Height	Width
Papillae on the introvert	About 0.0425 mm	About 0.0175 mm
Papillae at the introvert-base	„ 0.0425 mm	„ 0.0262 mm
Papillae in the middle region of body	„ 0.0425 mm	„ 0.0325 mm
Papillae in the posterior end of body	„ 0.0300 mm	„ 0.0275 mm

Neither hooks nor spines are found on the introvert. The tentacles are filamentous and are very numerous, estimating over four hundred. They are arranged in 20 radial double rows, each of which bears 10–16 tentacles.

The longitudinal muscle layer is continuous. The inner surface of the body-wall shows a pearly luster. Two slender retractor muscles (Pl. III, Fig. 12, rm) arise from the middle of the posterior one-third of the trunk and fuse together into one at the anterior portion of the introvert and cover the oesophagus (Pl. III, Fig. 12, o). A single stout spindle-muscle (Pl. III, Fig. 12, sm) arises behind the anus (Pl. III, Fig. 12, a) and its posterior extremity is set free from the body-wall. There are three slender fixing-muscles (Pl. III, Fig. 12, fm), of which two arise from the dorsal wall of the trunk and attach themselves to the first whorl of the intestinal convolution, while the remaining arises also from the dorsal wall, far distant anteriorly from the former, and is attached to the oesophagus. Well developed wing-muscles (Pl. III, Fig. 12, wm) are found upon both lateral sides of the rectum near the anus. The intestinal convolution (Pl. III, Fig. 12, ic), which coils around the spindle-muscle, consists of about 25 double spirals. It is posteriorly free from the body-wall. Numerous and sometimes branched Polian tubules (Text-fig. 5, pt) are present upon the Polian canal (Pl. III, Fig. 12, pc) which passes along the dorsal side of the oesophagus. The segmental organs (Pl. III, Fig. 12, so) which consist of two small sacs of a deep reddish-brown colour, hang free into the body cavity, and their external apertures lie slightly distant from and in front of the anus. A pair of gonads (Pl. III, Fig. 12, g) is found

lying along the base of each retractor muscle. There are two eye-spots on the ganglion, and there is a large diverticulum (Pl. III, Fig. 12, rd
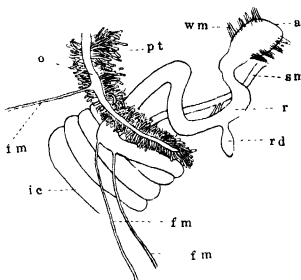
Text-fig 5 *Phascolosoma zenibakense* IKEDA Magnified view of a portion of the digestive canal. a, anus, fm, fixing-muscles; ic, intestinal convolution; o, oesophagus, pt, Polian tubules, r, rectum; rd, rectal diverticulum, sm, spindle muscle, wm, wing-muscles ×7.

and Text-fig. 5, rd) upon the rectum.

Localities. — Hokkaidô, Japan (1924, IKEDA). Off the coast of Asamushi Marine Biological Station.

Remarks. — The specimen here dealt with seems to agree fairly well with the description and figures given by IKEDA (1924, p. 29), but shows some differences indicated in the following table.

	IKEDA's description	The specimen from Mutsu Bay
Trunk	85 mm. in length 10 mm. in thickness	60 mm. in length 10 mm. in thickness
Introvert	much narrower than, but nearly as long as, the trunk	40 mm. in length 3.5 mm. in thickness
Papillae	about 0.05 mm. high about 0.025 mm. wide	about 0.04 mm. high about 0.03 mm. wide

	Ikeda's description	The specimen from Mutsu Bay
Tentacles	not less than 200, in about 20 regular rows	not less than 400, in about 20 regular double rows
Retractor muscles	arise from the middle of the posterior two-thirds of the distance between the anus and the posterior end of the body	arise from the middle of the posterior one-third of the length of the trunk
Intestinal convolution	consists of about 30 spirals	consists of about 25 spirals
Rectal diverticulum	absent	present
Eye spots	absent	present

Genus PHASCOLION Theel.

The longitudinal muscle layer of the body-wall is perfectly continuous. There is only one segmental organ. One or two retractor muscles are present. A single row of tentacles appears around the mouth, and numerous, recurved small spines often occur in a zone behind the tentacles. In most species, numerous attaching papillae, each of which is capped with one or more spines, are arranged in a broad band encircling the body. The body is generally twisted into spirals. The worms usually inhabit the shells of Gastropods or Scaphopods, but sometimes they also live in the tubes of Annelids or in those constructed by themselves.

7. Phascolion ikedai, n. sp.
(Pl III, Figs 13–17, Text-figs 6–9)

Many specimens were obtained by Professor S. Hózawa and Mr. S. Takatsuki from the muddy-bottom of Mutsu Bay at a depth of about 30 fathoms by means of a scrape-dredge. The worm lives in a state of commensalism with a Madreporarian coral, Stephanoceris carthausi Felix (Pl. III, Figs. 13–14). This worm was first found by Dr. Uchida in the coral above mentioned which was sent to him by Professor Hozawa for identification.

In the specimen (Pl. III, Fig. 15) selected as the type, the length of the trunk is about 7 mm., and the introvert is about two-thirds of the length of the trunk. The trunk is covered with a great number

of large and flat papillae. Those distributed on the anterior one-third of the trunk are exceedingly large, being 0.05-0.1 mm. high and 0.09-0.35 mm. wide, and form the attaching papillae, "Haftpapillen". On the introvert and at the introvert-base, the papillae are much smaller than those found in other regions of the trunk and each of them is closely beset with numerous polygonal plates. At the introvert-base the papillae (Text-fig. 7) are arranged in more or less regular rows and measure about 0.035-0.02 mm. in height and 0.08-0.015 mm. in width at the base. On the introvert the papillae (Text-fig. 6) are cylindrical in form measuring about 0.07-0 05 mm. in height and 0.027-0.012 mm. in width About 40-80 attaching papillae are scattered on the surface of the trunk Each of the papillae distributed on the convex surface of the trunk bears a large spine (Pl. III, Fig. 17) while that disposed on the concave surface is furnished with a small one (Pl. III, Fig. 16). The papillae which are placed near the mid-ventral line are entirely devoid of spines. Near the anterior end of the introvert, immediately behind the mouth, there is a group of small spines, one of which is indicated in Text-fig. 8. No tentacles can be detected on the introvert.

The longitudinal muscles of the body-wall form a continuous layer. Only one retractor muscle (Text-fig. 9, rm) with two short roots arises from near the posterior end of the body. The intestinal con-

Text-fig 6   *Phascolion ikedai*, n sp.
Side view of papillae on the introvert.
×360.
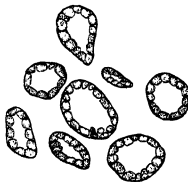
Text-fig. 7   *Phascolion ikedai*, n sp.
Surface view of papillae at the intro-
vert base. ×360

Text-fig 8   *Phascolion ikedai*, n. sp. A beak-shaped spine on the anterior end of the introvert. ×360.

volution (Text-fig. 9, ic) consists of a few irregular spirals. A large single segmental organ (Text-fig. 9, so) is found on the right side of the
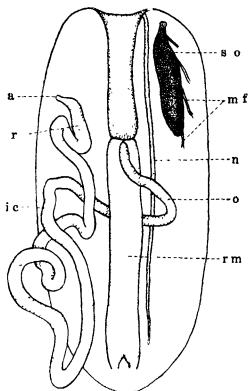
Text-fig 9 *Phascolion ikedai,* n. sp   Specimen dissected.
a, anus; ic, intestinal convolution, mf, muscle fibers, n, ventral
nerve-cord; o, oesophagus, r, rectum, rm, retractor muscle. ×14.

nerve-cord, and is fixed to the body-wall by the several stout muscle fibers (Text-fig. 9, mf). The aperture of the segmental organ is situated far anteriorly from the anus (Text-fig. 9, a).

Remarks. — This new species closely resembles *Phascolion collare* SELENKA et DE MAN in general features. But, however, it differs from the latter in the following points : — (1) The arrangement and form of the spines set on the attaching papillae on the concave surface of the trunk. (2) The situation of the aperture of the segmental organ. (3) The size of the body.

The most interesting point in regard to this new species is the fact that it lives in a state of commensalism with a Madreporarian coral, *Stephanoceris carthausi* FELIX. In regard to the commensalism between the Sipunculoid and the coral there have been reported by SEMPER (1872), MÖBELY (1881), ALCOCK (1893), BOUVIER (1895), SLUITER (1902) and IKEDA (1922), cases in several species of the genus *Aspidosiphon*. But there is no record in the case of the genus *Phascolion* except for IKEDA's paper. In that report, he has dealt chiefly with the ecological observations on the commensalism between these two animals (the specimens obtained from Sagami Sea). Concerning the identification of the species of the worm, he only suggested that it is undoubtedly a new species belonging to the genus *Phascolion*, concluding from the presence of a single segmental organ, the presence of the attaching papillae capped with large spines and the presence of other features characteristic to that genus. But the specific name was left undetermined.

In the specimen from Mutsu Bay, there is inserted a Molluscan shell between the worm and the coral as IKEDA (1922) has observed in the specimen taken from Sagami Sea. The greater part of the shell is dissolved and it seems to be difficult to determine the species, but judging from features of the remaining part of the shell there is no doubt that it is of a Gastropod species.

In most cases this worm lives in *Stephanoceris carthausi* FELIX together with a Polychaeta errantia belonging to the genus *Syllis*, more than 40 mm. long and 1 mm. broad. These facts were also observed by IKEDA (1922) in the specimens taken from Sagami Sea.

Genus DENDROSTOMA GRUBE.

The longitudinal muscle layer of the body-wall is continuous. 4–6 dendritic tentacular stems surround the mouth. Large hooks are scattered on the introvert. Two or four retractor muscles, and one spindle-muscle are present. There are two segmental organs, free from the body-wall excepting for the attachment base. The dermal papillae are small.

Key to the species of *Dendrostoma* found in Mutsu Bay.

I. Tentacles arise from four main stems. The papillae in the hooked

region on the introvert are equally short.

.... *Dendrostoma blandum* SELENKA et DE MAN.

II. Tentacles arise from six main stems. The papillae in the hooked
region on the introvert are found tall and short mixed.

.... *Dendrostoma hexadactylum*, n. sp.

8. **Dendrostoma blandum** SELENKA et DE MAN.

(Pl IV, Figs. 18–19; Text-figs 10–12)

Dendrostoma blandum, SELENKA et DE. MAN, 1883–1884, p 85, Pl I, Fig 9, Pl XI,
Figs 159–162, 1885, p 14; I IKEDA, 1904, p 53, Pl I, Fig 14, Pl IV, Figs
90–91, 1924, p 30, Pl I, Fig 2, A OSTROUMOV, 1909, p 319–324, W FISCHER,
1922 (2), p 18

I obtained a single specimen (Pl. IV, Fig. 18) at Tsuchiya on a
rock covered by seaweeds (*Sargassum* sp.) on August 29, 1927.

The trunk measures about 7 mm. in length and 3 mm. in thickness.
The introvert is nearly as long as, but much narrower than, the trunk.

The skin of the trunk is thick and brownish-yellow in colour, while
that of the introvert is thin and somewhat translucent, appearing
grayish-white. A smooth pigmented zone (Text-fig. 11, pz) lies in the
anterior end of the introvert behind the tentacular crown. The whole
surface of the skin is covered with small papillae, and in the centre
of each papilla there is a clear area surrounded by small granules.

The papillae in the middle region
of the trunk are flat and elliptical
in the surface view. They are
about 0.05 mm. in major axis and
0.035 mm. in minor axis in the larger
ones, while in the smaller the major
and minor axes are about 0.04 mm.
and 0.025 mm. respectively. In the
posterior region of the trunk they
become large and more or less
roundish in the surface view. On
the introvert just behind the hooked
region, the papillae are most pro-
minent measure about 0.09 mm. in
height and 0.05 mm. in width. In

Text-fig 10 *Dendrostoma blandum*
SELENKA et DE MAN. Frontal view of
the tentacular crown. ×20.

the hooked region (Pl. IV, Fig. 19) of the introvert there are small
pear-shaped papillae about 0.03–0.04 mm. high and wide. The hooks
(Pl. IV, Fig. 19) on the introvert are numerous, being 103 in all.
They are 0.1–0.2 mm. high and are of a blackish-brown colour. The
tentacular crown (Text-fig. 10) consists of four main tentacular stems
which are divided into eight tentacular arms. The tentacles spring
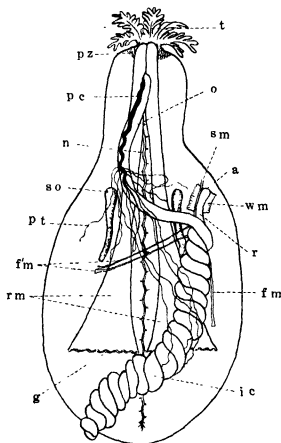
Text-fig. 11. *Dendrostoma blandum* SELENKA et DE MAN. Spe-
cimen dissected. a, anus; fm, fixing-muscles; g, gonad; ic, intestinal
convolution, n, ventral nerve-cord; o, oesophagus; pc, Polian canal,
pt, Polian tubule; pz, pigmented zone; r, rectum; rm, retractor
muscles; sm, spindle-muscle; so, segmental organ; t, tentacles; wm,
wing-muscles. ×8.

from both sides of each tentacular arm, and the number of the tentacles on each side is from ten to sixteen. The colour of the tentacles is grayish-white when preserved in alcohol.

The longitudinal muscles are continuous and the inner surface of the body-wall is shiny, but in the region of the roots of the retractor muscles the peritoneum shows some shallow and narrow longitudinal grooves. The retractor muscles (Text-fig. 11, rm) occur in a single pair, and are attached to the body-wall at the level of the posterior one-third of the trunk. A spindle-muscle (Text-fig. 11, sm) springs from the dorsal body-wall close to and in front of the anus (Text-fig. 11, a), and its posterior end is not fixed to the body-wall. There are three fixing-muscles (Text-fig. 11, fm, and Text-fig. 12, fm), two of which arise from the left side of the dorsal body-wall and are attached respectively to the oesophagus (Text-fig. 11, o and Text-fig. 12, o) and rectum (Text-fig. 11, r and Text-fig. 12, r), and the remaining one of which is broader than the others and starts from the right side of the body-wall in front of the root of the right retractor muscle and is attached to the rectum far posteriorly from the anus. The broad wing-muscles (Text-fig. 11, wm) are attached on both sides of the rectum near the anus. The intestinal convolution (Text-fig. 11, ic), consisting of about 20 spirals, is free posteriorly from the body-wall. The Polian canal (Text-fig. 11, pc) which passes along the dorsal side of the oesophagus is divided into a

Text-fig. 12. *Dendrostoma blandum* SELENKA et DE MAN. Magnified view of a portion of the digestive canal. a, anus; fm, fixing-muscles; ic, intestinal convolution; o, oesophagus; r, rectum; wm, wing-muscles. ×12.

tuft of long tubes near its posterior extremity. These tubes (Text-fig. 11, pt) entangle to a great degree with the intestinal spirals, retractor muscles and other internal organs. The short segmental organs (Text-fig. 11, so), of about one-third of the trunk length, are grayish-yellow in colour. Their external apertures are situated almost at the same

level as the anus. A pair of gonads (Text-fig. 11, g) is found along
the base of the retractor muscles. The rectal diverticulum is absent.
Eye-spots are seen on the ganglion as a pair of small pigmented spots.

Localities. — California ; Enoshima and Hokkaidô, Japan. In Mutsu
Bay it was formerly reported from Aomori by IKEDA (1904, p. 55),
and this time it was obtained at Tsuchiya.

Remarks. — The specimen seems to be identical with Dendrostoma
blandum which was first described by SELENKA et DE MAN in 1883–
1884 dealing with the specimens from Enoshima, Japan. In dissecting
the specimen from Mutsu Bay, however, several differences were revealed
which exist between the said specimen and those reported by SELENKA,
FISCHER and IKEDA. viz. SELENKA and DE MAN (1883–1884, p. 86)
reported that the tentacles arise from five or six main tentacular stems.
But afterwards, IKEDA (1904, p. 55) stated that : — " The tentacles are
given off from the free margins of the eight arms produced by the
dichotomous branching of each of the four main stems." And FISCHER
(1922, p. 18) states refering to this point that. — " IKEDA (1904, p. 53)
lieferte Ergänzungen resp. Berichtigungen zu der Beschreibung SELENKA's.
Dieser gibt nämlich an, die Tentakel entsprängen aus 5–6 Hauptstämmen.
IKEDA sah nur 4, die sich in je 2 Arme teilten. Ich konstatierte eben-
falls 4 Hauptstämme, die aber in je zwei grössere und zwei kleinen
Arme teilten so dass 16 Arme sichtbar waren. Die kleinen Arme
zweigten sich nur von einen der grösseren Tentakel ab. Die Arme
waren, wie IKEDA angibt, in ihren ganzen Verlaufe mit kurzen Ten-
takeln bedeckt. 16 Arme teilten sich weiter in je 4–5 Zweige, die
mit vielen Tentakeln bedeckt waren." IKEDA (1924, p. 31) again
reported on the same subject, basing his argumented upon the specimens
obtained abundantly from Hokkaidô, Japan, that : — " There are four
short tentacular stems, each of which divides into two longer branches.
This confirms my former statement on this same point for the specimens
from Aomori and Wakkanai. It must, however, be mentioned that
the two branches of a tentacular stem are often remarkably different
in size, consequently casual observers may fall into error concerning
the true manner of branching, or even the number of the tentacular
stems. According to SELENKA, there are present 5 or 6 main tenta-
cular stems ; but these may be rather anomalous cases of the branching
of the stems." While in the specimen from Mutsu Bay, as already

mentioned above, the main tentacular stems are distinctly four in number, and the each is divided into only two arms in the same manner as in the case of IKEDA's specimens from Aomori and Wakkanai. On the fixing-muscles it was reported by IKEDA (1904, p. 55) and FISCHER (1922, p. 18) that one of them is divided into two branches, while in the specimen from Mutsu Bay it is simple, not being branched. IKEDA (1904, p. 55) and FISCHER (1922, p. 18) noted the existence of a diverticulum upon the rectum, but such a structure was not found in the specimen from Mutsu Bay. Concerning the length of the trunk and introvert, the specimen from Mutsu Bay shows smaller dimensions than those reported by SELENKA and IKEDA as shown in the following table.

	Sp from Mutsu Bay	SELENKA's sp (1883–4, p 86)	IKEDA's sp. (1904, p 54)	IKEDA's sp. (1924, p 30)
Length of the trunk.	About 7 mm	Less than 25 mm	2.5 mm. (25 mm ?)	40–45 mm
Length of the introvert	About 7 mm	One-third of the body length		

9. Dendrostoma hexadactylum, n. sp.
(Pl IV, Figs 20–24, Text-figs 13–15)

Three specimens were collected from the different localities in Mutsu Bay. The first specimen (Pl. IV, Fig. 20) was taken by myself off the coast of Tsuchiya on September 16, 1927, by means of a dredge; the second (Pl. IV, Fig. 21) was collected at the coast of Tsuchiya from a rock covered by seaweeds by Mr. T. IMAI, in August, 1927; and the third was obtained at Urata by Mr. S. TAKATSUKI, in June, 1927, from a certain fissure of rocks near the tide-marks where a large number of oysters (*Ostrea circumpicta* PILSBRY) were found attached to the surface of these rocks.

The first specimen which was selected as the type of the new species is 55 mm. in length and is 10 mm. thick in 'a fully expanded state, the introvert being about one-third of the total body length. The second and the third specimens are some what smaller in size than the type specimen.

The animal in life is of a yellowish-brown colour on the general

body surface excepting for the introvert-base and the posterior end of the trunk which are of a little darker brown colour. At the anterior end of the introvert, immediately behind the tentacular crown, there is a ring (Text-fig. 14, pz) which is perfectly smooth and is irregularly coloured with light violet. The surface of the trunk when observed under the microscope, shows numerous transverse furrows forming numerous transverse bands encircling the trunk. In the posterior region of the trunk there occur also longitudinal furrows in addition to the transverse furrows above mentioned, and thus numerous small areas are formed. Each of these small areas is provided with a small papilla in the centre and the latter are circular in surface view. The papillae found in the middle region of the trunk (Pl. IV, Fig. 22) and in the region just behind the hooked region of the introvert, are elliptical in surface view. The papillae (Pl. IV, Fig, 23 and 24) situated in the hooked region of the introvert are cylindrical and not uniform in height, some being shorter and the others taller. The measurements of the papillae on the different regions of the body-surface are shown in the following table.

		Type (1st) sp	2nd sp	3rd sp
Height of papillae in hooked region		0.07–0.103 mm	0.055–0.095 mm	0.05–0.145 mm
Diameter of the base of the same		0.06–0.085 mm	0.055–0.057 mm	0.045–0.07 mm.
Length of major and minor axes of papillae in the middle region of the body.	Minor; Major	0.075–0.1 mm.	0.06–0.08 mm	0.065–0.095 mm
		0.05–0.075 mm	0.05–0.065 mm.	0.057–0.075 mm.
Length of major and minor axes of papillae in the posterior region of the body.	Minor; Major	0.08–0.107 mm.	0.075–0.1 mm.	0.07–0.1 mm
		0.07–0.105 mm.	0.065–0.1 mm	0.06–0.095 mm.

The hooks (Pl. IV, Fig. 23) on the introvert are very numerous, numbering over 260, and they measure 0.2–0.36 mm. in height. The tentacles (Text-fig. 13) are light reddish-brown in colour. There are six main stems of the tentacles, and the each is divided into 1–4 stems.

Each of these stems is again divided into branches in an irregular
manner. The finger-shaped tentacles are arranged chiefly on the
branches above mentioned.
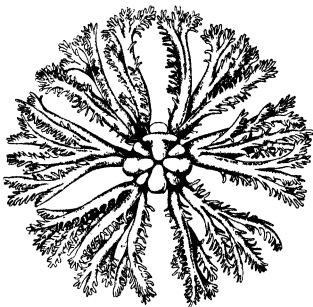
Text-fig. 13 *Dendrostoma hexadactylum*, n. sp. Frontal view of
the tentacular crown ×5.

The longitudinal muscle layer is perfectly continuous and the inner
surface of the body-wall is remarkably smooth and shows a pearly
luster. Two broad retractor muscles (Text-fig. 14, rm) spring from
the body-wall at the level of the posterior one-third of the trunk. A
spindle-muscle (Text-fig. 14, sm) arises from the body-wall in front of
the anus (Text-fig. 14, a), and posteriorly ends free from the latter.
The fixing-muscles (Text-fig. 14, fm) are three in number. They arise
from the dorsal body-wall of the middle portion of the trunk and at
a point far distant from the nerve-cord (Text-fig. 14, n). Two of
these muscles are attached respectively to the oesophagus (Text-fig.
14, o) and the rectum (Text-fig. 14, r), while the remaining is attached
to both the oesophagus and the rectum by means of its two rootlets.
These features concerning the fixing-muscles seem to vary to some

extent in different specimens. The text-fig. 15 (A, B and C) indicates
the variations found in the fixing-muscles in the specimens taken from
Mutsu Bay. A pair of broad wing-muscles (Text-fig. 14, wm) is
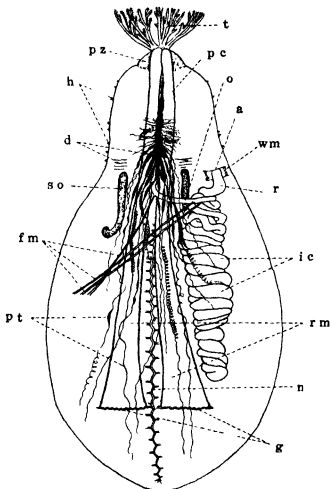
Text-fig. 14. *Dendrostoma hexadactylum*, n. sp. The type specimen
dissected. a, anus; d, short disepiments; fm, fixing-muscles; g, gonads;
h, hooks; ic, intestinal convolution; n, ventral nerve-cord; o, oesophagus;
pc, Polian canal; pt, Polian tubules; pz, pigmented zone; r, rectum; rm,
retractor muscles; so, segmental organ; t, tentacles; wm, wing-muscles.
×2.

attached to the lateral sides of the rectum near the anus. The Polian
canal (Text-fig. 14, pc) passes along the dorsal surface of the oesophagus
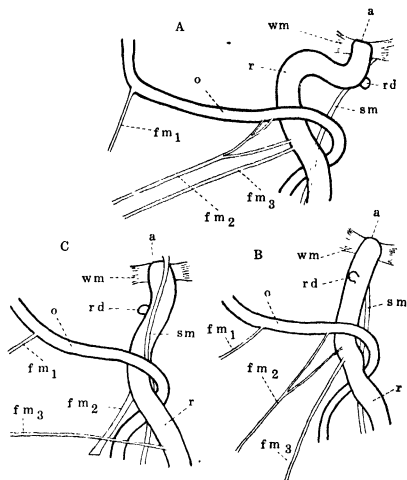
Text fig. 15. *Dendrostoma hexadactylum*, n. sp. Magnified view of a portion
of the digestive canal A, Type (1st) specimen, B, 2nd specimen; C, 3rd specimen;
a, anus, fm₁, fm₂, fm₃, fixing-muscles; o, oesophagus, r, rectum; rd, rectal diverti-
culum; sm, spindle-muscle; wm, wing-muscles. ×6.

and gives off a great number of long blind tubules (Text-fig. 14, pt)
on its way. These tubules are often spirally twisted and are greatly
entangled with the intestinal convolution and with other internal organs.

The segmental organs (Text-fig. 14, so), consisting of two short tubes of a grayish-yellow colour, are entirely free from the body-wall except for the anterior ends which are fastened to the latter. Their external apertures exist almost at the same level as the anus. Several narrow dissepiments (Text-fig. 14, d) are seen in front of the attachment base of each segmental organ, arranged transversally in a longitudinal line. These dissepiments seem to be identical with those found in *Siphonosoma cumanense* (KEFERSTEIN), though they are very broad and numerous in the case of the latter species. A pair of sexual organs (Text-fig. 14, g) occur along the base of the two retractor muscles. No eye-spot can be detected on the ganglion. A small globular diverticulum (Text-fig. 14, rd) is found upon the rectum.

Localities. — Tsuchiya and Urata.

Remarks. — This new species closely resembles *Dendrostoma blandum* SELENKA et DE MAN in size of body and in the internal structures, but, however, it differs from the latter in the height of the papillae found in the hooked region of the introvert, as well as in the features of the tentacular crown, viz., the number of the main tentacular stems is six in the present species, while in *Dendrostoma blandum* SELENKA et DE MAN, according to FISCHER and IKEDA, it is usually four.

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EXPLANATION OF THE PLATES.

PLATE I.

Fig. 1 *Sipunculus nudus* LINNAEUS. Natural size.

Fig. 2. *Siphonosoma mourense*, n sp The type specimen. Natural size.

Fig. 3 The same, dissected to show the anterior region of the trunk. a, anus, dr, dorsal retractor muscles, fm, fixing-muscles, ic, intestinal convolution, in, infundibulums of the segmental organs, kb, KEFERSTEIN's body, o, oesophagus, pc, Polian canal, r, rectum, sm, spindle-muscle, so, segmental organs, n, ventral nerve-cord, vr, ventral retractor muscles, wm, wing-muscles ×2 5

Fig. 4 The same A part of a row of the tentacles Greatly magnified.

Fig. 5. *Physcosoma japonicum* (GRUBE) Natural size.

Fig. 6. *Physcosoma glaucum*, n. sp Surface view of a papilla in the middle region of the trunk ×500

PLATE II

Fig. 7. *Physcosoma glaucum*, n sp The type specimen Natural size

Fig. 8. The same dissected. a, anus, dr, dorsal retractor muscles, es, eye-spot, fm, fixing muscle, ic, intestinal convolution, n, ventral nerve cord, o, oesophagus, pc, Polian canal, r, rectum, sm, spindle-muscle, so, segmental organs, vr, ventral retractor muscles, wm, wing-muscles ×4

Fig 9 *Physcosoma scolops* (SELENKA et DE MAN) Natural size

Fig 10 The same dissected. a, anus; dr, dorsal retractor muscles, es, eye-spot, fm, fixing muscle; ic, intestinal convolution; n, ventral nerve-cord, o, oesophagus; r, rectum; sm, spindle muscle, so, segmental organs, vr, ventral retractor muscles; wm, wing muscles ×4

PLATE III

Fig. 11. *Phascolosoma zembakense* IKEDA. Natural size

Fig 12 The same dissected. a, anus, fm, fixing-muscles, g, gonad, ic, intestinal convolution; n, ventral nerve-cord, o, oesophagus, pc, Polian canal with Polian tubules; r, rectum; rd, rectal diverticulum, rm, retractor muscles; sm, spindle-muscle; so, segmental organs, t, tentacles; wm, wing-muscles ×1.5.

Fig. 13. and Fig. 14. *Stephanocerus carthausi* FELIX ×2

Fig. 15. *Phascolion ikedai*, n sp. The type specimen ×6

Fig. 16. The same. Spines on the concave surface of the trunk. ×90.

Fig. 17. The same Spines on the convex surface of the trunk. ×90

PLATE IV.

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Fig. 21. The same. The second specimen. Natural size
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         c, cuticle, cm, circular muscle layer; dg, duct of the subdermal gland;
         e, epidermis; lm, longitudinal muscle layer; pa, papilla; pe, peritoneum;
         sg, subdermal gland, ss, subepidermic space.

Study of *Euryale ferox* SALISB. V.

On some features in the physiology of the seed with special respect to the problem of the delayed germination.

BY

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(With Plate V and 4 Text-figures.)

CONTENTS

1 INTRODUCTION AND HISTORICALS

A few years ago, the author happened to obtain some seeds of a special type of *Euryale ferox* SALISB., studied their germination behavior to learn the marked delay in germination, and wrote a brief paper in which the suggestion was expressed that the seeds of the plant may obtain full germinative capacity after a lapse of two winter seasons in their natural habitat after their apparent ripening, i. e., the time of normal separation from the mother plant (OKADA, 1925). The number of the seeds then employed was too small to carry on a systematic study of the matter, and the author was able to reach no conclusive result beyond the mere suggestion as to the mechanism of the dormancy. The study has been since continued, during the course of these several years, depending upon the supply of material from the same origin as in the previous study, and some of the features concerning the problem have been more or less elucidated, if not entirely satisfactorily. Although the experimental data hitherto accumulated are evidently far from complete in solving the essential problem of the phenomenon, the author feels that the record of these data should be conveniently arranged in some definite form, in order that the principle for further study may be established.

The present study was, as already mentioned in the preliminary report of 1925, first commenced under the guidance of Prof. Dr. MIYOSHI with the materials he obtained from Zyûnityôgata. After the removal of the author to Tôhoku Imperial University at Sendai, the study has been continued there up to this date. It is with great pleasure that the writer wishes to express his indebtedness to Prof. Dr. MIYOSHI under whose kind guidance the study was commenced. As for the supply of the material, the author is especially grateful to Mr. MATOBA and Mr. OTAYA, without whose kind help the progress of the study would have been much retarded or even impossible. The expense of the study since 1927 was partly defrayed by the Subsidy to Promote the Study of Natural Sciences, endowed by the Department of Education, for which the author is also very much indebted. Acknowledgement is, furthermore, due to all those who, more or less, either directly or indirectly, have helped the author to carry out the study, and to bring it to its present state of progress.

The embryo of the seed plant is, in the early stage of its develop-
ment, a parasite to its mother plant, the untrition of the former being
wholly dependent on the supply from the latter. The embryo thus
brought up is, however, destined to be delivered sooner or later,
together with its accumulated foodstuff and incasing structure for
protection, from the mother plant. The whole structure, i. e., the
embryo with its attribute, thus separated normally from the mother
plant represents conventionally a full ripe seed (or fruit in the case
of the inclusion of the grown carpellary wall). Now, it is quite a
remarkable fact that, when such a full ripe seed is subjected to a
sufficiently favorable germinative condition, the reaction displayed is
far from uniform in each case. Seeds of some plants do respond
quite readily and result in a high percentage of germination, while,
on the other hand, those of some other plants are highly refractory
and their percentage of germination in the period directly after the
harvest is quite low or even nil. For the seeds of the latter category,
ordinary germinative conditions which are accepted to be valid enough
to induce germination in many cases are totally ineffective. With all
the favorable conditions combined, viz., optimum temperature, ample
supply of water and oxygen, and the presence or absence of light
according to their light requirement, we can either obtain no germi-
nation at all, or induce germination in a limited number of seeds.
Reasonably high percentage of germination can be attained only after
a lapse of some period. In short, such seeds are germination incapable
when freshly harvested, notwithstanding their apparent maturity, and
they attain the germinative capacity only after an aging period under
a certain condition. The span of this period is the so-called dormant
period, or by some authors the period of after-ripening, and this mode
of germination is called a delayed germination (or distributed germina-
tion in case of chronical irregularity).

Seeds provided with such a property are not of exceptional occur-
rence. It seems even a majority are possessed of such tendency in
some degree. One may consult with respect to this point, for instance,
the tables by KINZEL (1913) or the work of HOWARD (cit. CROCKER,
1916, p. 99).

According to the citation by SHULL, some of the earliest works
along this line of the problem are to be found in NOBBE (1876),

NOBBE and HÄNLEIN (1877), HÄNLEIN (1880), etc., in which are treated
the germinative behaviors of various agricultural and weed seeds, and
are mentioned the frequent occurrence of markedly delayed or distri-
buted germinations, as well as some considerations about the cause of
the phenomenon (SHULL, 1912, p. 454).

KIENITZE (1880) noticed that the seed of ' Weisstanne ' and ' Buche '
do not germinate in the autumn of the harvest but in the next spring,
and also that the seeds of ' Eschen ', ' Hainbuchen ' and ' Zirben ' show
examples of distributed germination.

WINKLER (1883) reports on the observation on the delayed and
distributed germination of *Tithymalus cyparissias*. He mentions also
Reseda lutea, *Dianthus armeria* and *Malva moschata* as further
examples.

In 1886, a brief paper was issued by LUDWIG, reporting the dor-
mancy of the seeds of *Mayaca fluviatilis*.

A little later, BATALIN's paper (1889) appeared, which reports that
the germination of the seeds of ' Roggen ' and some other species can
be favored by desiccation by heat. Similar effect of cool storage on
the seeds of ' Hafer ' is also mentioned.

SAUVAGEAU (1894), in a paper treating of the biology of the
Potamogetonaceae, noticed the dormancy of the seeds of *Potamogeton
lucens*, *P. crispus*, *P. perfoliatus*, *P. pectinatus*, and *P. natans*, of which
the last species is especially remarkable for a dormant period of three
years.

ARTHUR's paper in the next year (1895) reports for the first time
the noticeable distinction in the germinative behaviors between the
upper and the lower seeds of *Xanthium*, the former being the more
refractory.

WIESNER (1897) noticed that the seeds of *Viscum album* necessitate
a rest period of half a year when kept under the natural condition,
while the seeds of allied species in the tropics germinate readily.
Studies on the germinative condition are also given.

MAZÉ (1902) gives a note on the forcing effect of desiccation and
heat on some dormant seeds.

In 1906, CROCKER made progress along this line of study in his
paper dealing with the mechanism of the dormancy. He noticed first
that the delayed germination of the *Xanthium* seed is due to the

seed coat impervious to oxygen. He elucidated also that the cause of the dormancy of *Axyris, Abutilon, Chenopodium, Iris, Avena, Plantago* and *Thlaspi* is due mostly to the coat character. As for the *Crataegus* seeds, the dormancy seems to be caused by the property of the embryo.

In the next year, FISCHER published the result of his study with *Alisma, Sagittaria, Potamogeton* and other water plants. Based on the observation that the seeds of these plants, when kept in pure water, remain dormant for several years without any sign of germination, while they germinate readily in fermenting water, he carried on a series of experimental studies, come to the conclusion that the H⁺ or OH⁻ ion in the water influences the plasma of the embryo and activates them.

This conception of FISCHER was as soon encountered by the criticism of CROCKER who issued a paper (1907) in which he gives a contrary notion that the dormancy of the above said water plants is not of embryonic but of seed coat character, for those seeds germinate readily in case of either the total or partial elimination of the coat without any ionic influence. Later, in 1914, in his joint work with DAVIS, a more detailed study on the dormancy of *Alisma plantago* was published, indicating clearly that the delayed germination in this case is caused by the mechanical interference of the seed coat which inhibit the free imbibition of water by the embryo.

In a paper by KINZEL in 1908, which is mainly devoted to the study of the light relation of germination, we can find also an example of delayed germination observed with the seeds of *Allium suaveolens* (p. 111).

SHULL (1909) measured the oxygen requirement of the *Xanthium* seed, noticed the distinction between the upper seeds and the lower ones, and concluded that, besides the difference of the coat character discovered by CROCKER (1906), the embryonic difference in the oxygen requirement is also responsible for the difference in their delayed germination. Later, in 1911, 1914 and 1923, he and his co-worker published the result of the studies further concerning the oxygen requirement, temperature relation, respiration, and the catalase activity of the *Xanthium* seeds, and discussed their relation to the delayed germination.

GASSNER's papers in 1910 deal with the seeds of *Chloris ciliata*,
Ch. distcophylla and *Stenotaphrum glabrum* which after-ripen by dry
storage. It is noticed that the presence of light is a necessary condi-
tion for their germination and this influence is of embryonic character.

SCHWAPPACH, in 1911, reported on the low percentage of germina-
tion of the *Abies* seeds freshly harvested, and methods of forcing them.

BECKER (1912) studied mainly the seeds of *Compositae*, and learned
that the dormancy of the seeds is due to the coat which is impervious
to oxygen, an important stimulant to the embryo.

In the same year, a paper was issued by DAVIS and ROSE con-
cerning the *Crataegus* seeds. They distinguished such cases of dormancy
as are due to the coat characters from those in which the embryo
itself requires some period of after-ripening, a distinction which is
often overlooked. They demonstrated that the dormancy of this plant
is due to the embryonic character, and that the after-ripening means
the necessary protoplasmic changes antecedent to germination. Accord-
ing to their opinion, it is more rational not to include the disinte-
gration or other modification of the incasing structures in the term
after-ripening. The forcing method is treated also in the paper.

ECKERSON's paper in 1913 mainly concerns the chemical change in
the course of the after-ripening of *Crataegus spp.* The increase of
acidity, water holding power, catalase and peroxidase, decrease of fat
and appearance of oxidase and sugar were established.

KINZEL, who had for many years devoted himself to the investiga-
tion into the effect of light and frost on the germination of seeds,
consummated the obtained results in a publication in 1913, where are
tabulated the germinative behaviors of various seeds over a long period.
We can find many examples of delayed or distributed germination
there.

In 1914, ATWOOD studied the delayed germination of the seeds
of *Avena fatua* and demonstrated that the after-ripening process in
this plant means the change in the oxygen permeability of the seed
coat on the one hand and the change (especially the increase of acidity)
in the inner part, on the other.

ROSE in 1915 devised an apparatus to effect mechanical abrasion
on the coat of hard seeds to eliminate the cause of delayed germina-
tion due to the seed coat character. The germination incapability of

various economic seeds is also treated in the paper.

An explicit review on the mechanism of dormancy of seeds was published in 1916 by CROCKER where the fundamental features of the matter are discussed. Classification of the cause of the dormancy is given.

The same author (1918), in a joint work with HARRINGTON, reported on the studies on the catalase and oxidase activities of seeds. There are treated in this paper the relations of these enzymes with dormant seeds of different types.

A paper by ROSE in 1919 concerns the after-ripening process of the seeds of Tilia, Sambucus and Rubus. It is proved that the necessary change is embryonic in Tilia, while the seed coat character is responsible in Rubus. The mechanism of the delayed germination of Sambucus is left undetermined.

JONES (1920) studied the physiology of the maple seed and established a marked difference in the germinative behavior exhibited by species of the same genus Acer. It is reported in the paper that the sugar maple requires a period of after-ripening, which is of the embryonic property. Chemical change during the period is also given.

Two papers by PACK in 1921 concern mainly the chemical change in the after-ripening period of juniper seeds. In the latter paper, the change of fat is fully studied.

In 1923 a paper was published by HARRINGTON and HITE concerning the dormancy of the apple seed, which is caused by the embryonic character

HELMS and JÖRGENSEN reported in 1926 that the hibernated seeds of Betula have the temperature minimum for germination much depressed in comparison with those freshly harvested.

SCHAUMANN's paper in 1926 deals with the noted delay in germination of Alisma plantago. It is recognized that the hindrance to prompt germination is mainly due to the mechanical influence of the seed coat, and that the elimination of this drawback is effected in the natural condition more by the alternation of temperature than the effect of acid in the medium.

WEISS (1926) studied the germination of the seed of Betula populifolia, to learn that the increase in the germination percent and the depression of the temperature minimum for germination can be

effected by after-ripening in cold storage. Later, in 1929, JOSEPH extended the study to other members of the genus and proved similar facts.

DAVIS's paper in 1927 reports of the study with the seeds of *Cornus florida*, *Sambucus canadensis*, and *Berberis Thunbergii*. The after-ripening process of the first species which is of embryonic character, was mostly studied. As for the second species, it is proved that some seeds are dormant while others are not. *Berberis* seeds seem not to be dormant. In the same year, a brief paper was issued by CROCKER indicating that the after-ripening of some Rosaceous seeds can be accelerated through stratification at low temperature.

So far I have given a review of the literature which mainly concerns the general aspect of dormancy of seeds, its occurrence, embryonic change, mechanical resistance of the coat and oxygen impermeability. Another important cause of delayed germination is found in the incomplete development of embryo in the seeds freshly harvested. Concerning this phenomenon one may consult the work of GOEBEL (1922, pp. 1211–1214), where examples and some literature are mentiond. IVES (1923) also reports of another example with respect to *Ilex opaca*, whose embryo is still in a rudimentary state at the time of harvest. He concludes that "this immaturity of embryo constitutes the main factor in the delayed germination." (pp. 73–74).

Lastly, delayed germination due to the water impermeability of the seed coat must be mentioned. This fact attracted attention quite early, and in some of the papers above cited, studies with respect to this problem are also to be found (CROCKER, 1906, pp. 280–81, ROSE, 1915). In EWART (1908), GUPPY (1912, p. 585) and others one can obtain further knowledge. The seeds of this category represent the so-called hard seeds whose coats are impervious to water when freshly harvested. During the course of the storage, denaturation takes place to permit the intrusion of the water, which induce the act of germination. If, however, the hindrance is eliminated by some artificial means, prompt germination is secured. The following methods are hitherto reported: —Mechanical abrasion or total elimination of the seed coat (MICHALOWSKI, 1894; JARZYMOWSKI, 1905; BERGTHEIL, 1907; ROSE, 1915; OHGA, 1927; PUSHKAREW and MOTRENKO, 1927), application of hot water (WERNICKE, 1895; JARZYMOWSKI, 1905; HONING, 1917), carbonization with sulfuric acid (ROSTRUP, 1898; TODARO, 1901; HILTNER and KINZEL,

1902; JARZYMOWSKI, 1905; BERGTHEIL, 1907; LOVE and REIGHTY, 1912; ROSE, 1919; NELSON, 1926; OHGA, 1927; PUSHKAREW and MOTRENKO, 1927; JONES, 1928), freezing (MIDGLEY, 1926), application of high pressure (DAVIES, 1928) and treatment with alcohol (VERSCHAFFELT, 1912).

It may be mentioned here in connection with the problem of delayed germination, that the germination incapability due to the light requirement of the seeds (e. g. light-requiring seeds in a dark germinator or dark-requiring ones under illumination) is treated by some authors (SKENE, 1924, p. 426) to represent a case of dormancy. I have omitted here to deal with the matter according to the opinion of CROCKER (1916, p. 119).

Delayed germination in the *Nymphaeaceae*. As referred to in the above lines, KINZEL reports in his work "Frost und Licht", a large number of experimental data concerning the germination of the seeds of divers species, among which we can also find the cases with *Nymphaeaceae*. There are mentioned the cases with *Nuphar luteum*, *Nuphar advenum*, *Nymphaea alba*, and *Nymphaea Lotus* (Table 3 and pp. 19-20). The seeds of all these species show more or less irregular or distributed germination, that is to say, the majority of these seeds require some period of after-ripening, during which time, according to KINZEL (p. 148), internal chemical changes are presupposed to occur.

Reports of similar observations are sporadically found in literature. For example, CONARD (1905, pp. 106-107) mentions the irregular germination of *Nymphaea coerulea*, *N. zanzibariensis*, and *N. elegans* × *N. zanzibariensis*. He cites in the same place WATERS' report (1886) on the same phenomenon in *N. odorata*.

ABER (1920, p. 36), too, observed that the seeds of *Nymphaea lutea* germinated to some extent after the first winter following the time of ripening, and the remaining majority sprouted after the second winter, i. e., after the dormant period of eighteen month.

OHGA (1927, pp. 16-17) states in his paper, concerning the germination of century old seeds of *Nelumbo*, that the seeds of this plant are provided with an incasing structure impervious to water so that they must be forced to germinate by eliminating the hindrance; as by carbonizing with sulfuric acid or by some mechanical treatment.

A paper by JONES (1928) also treats the dormancy of the

Nelumbo seeds caused through their hard coat and the method of overcoming it by mechanical treatment or application of sulfuric acid.

As for the seeds of *Euryale ferox*, ARCANGELI, who studied various features of this plant in detail, has already reported in his papers of 1887 and 88 that they are quite irregular in germination.

I have suggested in may first brief paper concerning the germination of *Euryale* that the seeds of the plant may need, in their natural habitat, a period of after-ripening of two winters before they are capable of germination. This view was soon contradicted by MIKI (1927, p. 107), who, studying the biology of water plants in the Ogura Lake, noticed that *Euryale* seeds often germinate readily after a lapse of a single winter season under outdoor conditions. Mr. NAKAJIMA of this Institute, also informed me of his observation of the same fact. At the time of the publication of MIKI's paper, I was engaging in the continued study of *Euryale* seeds, and I also, came to the conclusion that it is not absolutely necessary for the seeds to after-ripen for two winters, or that it is fairly natural to observe their germination during the first spring following the time of ripening. At any rate, the fact that they generally do necessitate a more or less prolonged period of after-ripening, is not to be denied. Herewith I shall proceed to report the results of my own experiments concerning the problem.

2 MATERIAL OF THE STUDY.

All the material for the present study was, except when otherwise stated, obtained from the Zyûnityôgata in Toyama Prefecture. In the experimental data recorded in the following sections, the materials employed are always indicated by numbers designating the dates of their collection. Below is the table of these numbers with their corresponding dates of collection.

Lot number	Date of collection
5	Oct. 10, 1925
6, 61–69, 610	Oct. 14, 1926
7, 711–740	Oct. 17, 1927
8	Apr. 15, 1928
80, 811–821	Oct. 12, 1928

Of the above lots, those of nos. 5, 6, 7, 8 and 80 consist of seeds from many fruits mixed together, that is, they did not originate from separate individual fruits. On the other hand, nos. 61–69, 610, 711–740, 811–821 each indicate a lot from a single individual fruit. This discriminate treatment of seeds naturally resulted quite a troublesome work in experimenting, and may seem somewhat too scruplous or almost cumbersome. But in some cases in the present study, such discrimination cannot be dispensed with, especially when the total number of materials in experiment is limited to a few, as Yoshii (1925, pp 122–134) shows clearly the importance of accounting for the fruit individuality.

3 STRUCTURE OF THE SEED

This subject was studied as early as in 1888 by Arcangeli, and a little later Weberbauer (1894) treated the same matter in his paper on the seed anatomy of the *Nymphaeaceae*. Weber (1907), too, dealt with the structure of the seed coat in comparing the fossil form with the living one. My own observation of the samples from Zyûnityôgata coincides in the main with those of the former workers. But some points of deviation, though of minor importance, were also noticed. Moreover, as it may not be superfluous to give a general outline of the morphological characters before proceeding further to the discussion of the physiological properties, the matter will be treated here in brief.

General features. The seed of *Euryale* from Zyûnityôgata is globular in form. The micropyle and the hilum are closely apposed together at one end of the seed, that is, the seed is anatropous. There is a patch of the seed coat around the micropyle, so differentiated as to form a sort of lid or operculum (Okada, 1928, 1 and 2).

Seed coat. The seed when delivered from a ripe fruit, is covered with a two layered arylar envelop. As this structure has only little concern with the germination phenomenon, we will proceed directly to the inner part, or the seed coat proper. This part represents a very hard object, and assumes a brown color when fully ripe, tending to be stained a black shade more and more when kept in swamp mud for a long period of time, perhaps a parallel phenomenon to the

staining of *Trapa* seeds noticed by MOLISCH (1926, p. 49). The thickness of the coat measures 1 to 1.5 mm., exclusive of the raphe. Differentiation into the following four layers are recognized (Text-fig. 1).

    A.   Epidermal layer   B.   Sclerenchymatous layer
    C.   Non-lignified layer   D.   Cork layer
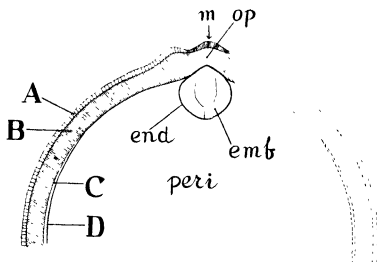
Text-fig. 1 Structure of seed (diagrammatized). A, B, C and D correspond to the respective four layers of the seed coat explained in the text end, endo-sperm, emb, embryo, m, micropyle, op, operculum, peri, perisperm

The relative thickness of these layers is somewhat variable according to the direction of the section. For example, at the equatorial section (assuming the micropylar end as the pole), on the opposite side to the raphe, the following ratio was obtained : — A : B : C : D=11 : 55 : 6 : 1, the real thickness of the whole testa measuring about 1.46 mm.

A Epidermal layer (" Hartschicht " of WEBERBAUER) (Text-fig. 1, A, Text-fig. 2, A)

This consists of a single layer of palisade cells, which extends all over the surface of the seed except the hilum. The cell wall is distinctly lignified (tested with phloroglucin solution plus hydrochloric acid), and is decidedly more thickened at the top and the bottom than at the

lateral side. The cell wall at the top is, not simply plane, but forms
a characteristic external protrusion, which gives a minute warty process
to each cell. These cells lack such complication on or near to the
operculum. *Lamellar structure is more or less accentuated in the wall*
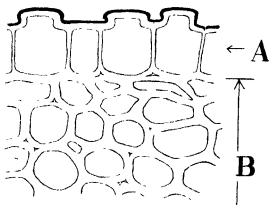
Text-fig 2 Epidermal layer (A) with a portion of the underlying
layer (B) ca 230×.

at the top, where the uppermost layer is deeply pigmented and assumes
a little higher degree of resistance to concentrated sulfuric acid. This
part shows, however, no affinity to Sudan III. Still further externally,
a covering surface of minor thickness is sometimes observed which is
stained violet with chlorzinc iodine. WEBERBAUER (1894, p. 221)
distinguished four layers in this last part, but I was unable to recognize
the differentiation.

        B. Sclerenchymatous layer (Text-fig 1, B, Text-fig. 2, B)

    Decidedly lignified, cell wall quite thick. The shape of the com-
ponental cell is rather isodiametrical towards the surface of the seed
and tends to be flattened radially in the deeper layer. The fibro-
vascular bundle runs mainly through this layer, being introduced
through the hilum. At the opercular region, below the epidermis, is
found the extension of this layer (Text-fig. 1, op); it may be noted
that this layer here is quite modified, the componental cell being
rather thin walled and not lignified. Thus this region assumes a

distinct contrast to the surrounding part and separates itself readily
as a part of the free operculum.
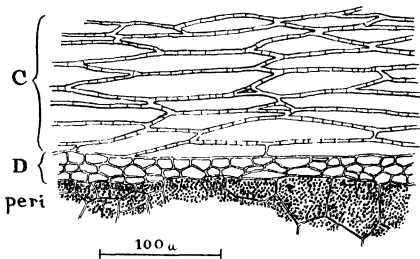
Text-fig 3 Non-lignified layer (C), cork layer (D), and a portion of peri-
sperm (peri) ca 350×

C   Non lignified layer (Text-fig 1, C, Text-fig. 3, C)

This layer is rather thin and is entirely lacking in the perimicro-
pylar region (as far as some 50° from the micropyle). This layer is
separated quite readily from the superposing sclerenchymatous layer,
and remains attached to the perisperm in case of artificial decortication.
The distal parts of the ramified fibrovascular bundles impress their
courses over the surface of this layer. As for the lignification, this
layer seems to be almost free from it except at the deepest part.
The cell walls seem to be slightly suberized as they are but faintly
stained with Sudan III. Moreover, it is provided with a quite high
grade of resistance to concentrated sulfuric acid, and stains brown
when treated with chlorzinc iodine.

D. Cork layer (Text-fig. 1, D, Text-fig. 3, D)

Distinct suberization is demonstrated by staining with Sudan III.
(This layer exhibits also a shade of pink colour when treated with

phloroglucin solution with hydrochloric acid). Extends all over the perisperm. Extremely thin, and particularly so at the perimicropylar region, corresponding to the region devoid of the preceeding layer C, where the suberization is reduced to a thickness of a single cell wall.

Perisperm. This part occupies the bulk of the inner body. It consists of larger cells filled with starch grains.

Endosperm. This part is reduced to an insignificant membranaceous structure around the embryo. The cells are characterized by their fatty inclusions which are densely stained with Sudan III.

Embryo. This is, too, a minute structure, being set in a position directly facing the micropyle. It measures about 2.3–2.9 mm. along the axis and almost equally wide across. Thick cotyledons in pair and a plumule with the leaf anlages are already differentiated. The

TABLE 1. Size, weight and dry matter content of the parts of the seed.

Whole seed	Height	13.25 mm
	Width	12.30 mm.
	Width/Height	92.91 %
	Fresh weight	1.284 g
	Dry weight	0.6558 g
	Dry wt./Fr wt	51.0%
Seed coat	Fresh weight	0.6734 g.
	Dry weight	0.2934 g.
	Dry wt /Fr. wt	44.13%
Perisperm	Fresh weight	0.6015 g.
	Dry weight	0.3579 g
	Dry wt /Fr wt	59.66%
Embryo	Length	2.75 mm.
	Fresh weight	8.94 mg.
	Dry weight	2.57 mg.
	Dry wt./Fr wt.	28.8%
Proportion of the parts (in fresh wt.)	Whole seed	100.
	Seed coat	52.43
	Perisperm	46.86
	Embryo	0.71

hypocotylar part is extremely reduced. The microchemical characters will be treated in other place in connection with the phenomenon of after-ripening.

The relative size, weight and dry matter content of the parts of the seed. Measurements were made with seeds freshly harvested with respect to the size, weight and dry matter content. The averages of the values obtained are shown in Table 1, which may help to give some notion on the matter, so far at least as the proportion of each part is concerned. Records of actual measurements from which the table is derived are given at the end of the paper.

On the criterion of the germination. The mode of germination was previously reported in my preliminary paper (OKADA, 1925) and need not be repeated here. It may, however, be appropriate at this place to mention that those seeds of which the hypocotylar end come into sight by pushing aside the operculum, are accounted for to be germinated. This criterion is held throughout the present work.

i DELAYED GERMINATION

Although the precise length of the period is not yet determined, the fact itself cannot be denied that the seeds of *Euryale* are germination incapable or at least they are highly refractory to the germinative conditions for a certain length of time after harvest, if they are apparently fully ripe. Such a property is suggested as soon if we take into consideration the state of the matter in their natural habitat. We know on the one hand, that the seeds of *Euryale* mature in general in October (in the northern region of Japan) under the water, are delivered as soon as the mother fruit wall is disorganized, and sink to the water bottom. On the other hand, the first germination of the seeds is observed to take place at the middle of April. Now, if we consider the environmental conditions there, we can not detect any decided inferiority of the germinative condition in October as compared with that of April. To make sure of this opinion, we shall consider here the germinative conditions in the natural habitat. Firstly the supply of moisture is out of question in this case, for the *Euryale* seeds always lie under water. Secondly, the water temperature is recorded to be higher in October than in April at Zyûnityôgata (In

1928, Apr. 10, 15°; Apr. 25, 17°; May 10, 21°; Oct. 10, 21°; Oct. 25, 19'; Nov. 10, 12°. Observation at 11, A. M., Water bottom). Thirdly, the oxygen supply must be very poor in both cases so far as the mud under water is concerned. Furthermore, the quantity of oxygen requisite for the germination of the *Euryale* seeds is quite limited (vide infra, section 5, c). Lastly, the seeds are indifferent to the effect of light as is shown also in a later section (section 5, b). Taking these points into consideration, we have no factor in the environmental condition, better fitted to suppress the germination of the seed in October. The cause of the delay is then to be accounted for the properties of the seeds themselves; they must be changed in some way or other during the period of the so-called after-ripening, if the changes do not necessarily concern the embryo proper. Only after the process is completed, the seeds acquire the capability of prompt germination.

The above theory is further verified by the behavior of *Euryale* seeds in the laboratory. I have treated over 10,000 seeds during these five years, none of which I have observed to germinate in the laboratory room in the period directly after harvest, although the room temperature is sufficiently high (Average of daily maxima and minima in the latter half of October : — 1925, max. 15°.2, min 11°.8 ; 1926, max. 14°.9, min. 11°.8 ; 1927, max. 14°.7, min. 11°.8 , 1928, max. 18°.1, min. 14°.3).

Furthermore, the application of even higher temperature does not effect the germination of the freshly harvested seeds. Below is an example of such : —

Experiment in 1927. Material, 100 seeds in total (10 seeds each from 10 different fruits, nos. 711–720). Temp., 25°. Light, excluded. Medium, 0.1% KNOP's solution Duration of experiment, Oct. 29, 1927 to Feb. 20, 1928. Result, no germination.

An entirely different result was obtained with seeds after-ripened in their natural habitat, i. e., in the mud of the water bottom. In the spring of 1928, on April 18, seeds were collected from among the mud of water bottom at Zyûnityôgata (lot no. 8). Of these after-ripened seeds, 104 out of 369 (28.2%) germinated within three days though the temperature was carefully controlled not to rise above 15°. It is shown in a later section that this lot of material not only exhibited

a high percentage of germination when the temperature is raised, but
also proved to be germination capable at the temperature in the
refrigerator (2°–8°). The reduction in the requirement is evident.

All these observed data indicate the necessity of after-ripening for
the germination of the *Euryale* seeds. As for the length of the
period, it is easily conceivable that the environmental condition may
greatly influence the length. The following table (Table 2) gives an

TABLE 2. Delayed germination of the *Euryale* seeds.

Material, seeds of autumn of 1926, sown directly after the harvest
Germinator, pot 70 cm wide and 30 cm. deep, filled with mud and
water A, in a hot house, B, in "Osakamuro" (a kind of covered
space, not specially heated).

	Lot	Number of seeds tested	Germination in		
			Oct., 1926– May, 1928	June 1928	July 1928
A	62	10	no germination	8	no germination
	63	4		0	
	64	4		2	
	66	10		8	
	67	4		0	
	69	4		4	
	610	4		2	
	Total	40		24	
B	62	10	no germination	6	4
	63	4		2	2
	64	4		0	0
	66	10		10	0
	67	4		2	2
	69	4		2	0
	610	4		2	2
	Total	40		24	10

example where the considerable length of the after-ripening period is
indicated.

It is demonstrated that the seeds sown in mud under water remain
inactive in the first spring following the harvest, both in a hot house
and in an "Osakamuro". In the second spring, the majority sprouted
as much as 85%. The remaining minority show no more signs of
mobilization in the third spring i.e., in the spring of 1929. Their
later fate is now under study.

A few years ago, I encountered a similar phenomenon which induced me to assume that the *Euryale* seeds germinate in general after two winters of dormancy under the conditions of their natural habitat. As is already referred to in the above section, MIKI's paper in 1927 (p. 107) and Mr. NAKAJIMA's personal information indicate that my former view must not be generalized. They observed *Euryale* seeds germinating in the first spring after the harvest. Their data are, however, obtained with seeds of *Euryale* of different origins than that of my study, which circumstance induced me to study the germinative behavior of *Euryale* seeds from various localities. These comparative studies resulted in showing that, 1) *Euryale* seeds of other origins than Zyûnityôgata do germinate to some degree in the first spring, but 2) majority of seeds do not respond until the second spring. The data in detail will be reported in another place, as the present paper is mainly related to the study of the Zyûnityôgata form. In connection with this point, it was found furthermore that if an ample supply of material is available in the study, we can obtain sometimes such seeds among the Zyûnityôgata origin as are able to commence germination without two winters of after-ripening. Table 3 represents an example where the germination of the seeds of the Zyûnityôgata species is observed to take place through one winter of after-ripening period.

In the above experiment, I obtained as high a germination percent as 20% in the first spring after the harvest. The deviation from Table 2 cannot be overlooked. The distinction in their treatment, however, seems rather trivial. These circumstances suggest that the after-ripening of *Euryale* seeds is rather a delicate process, slight modification of the environmental factors causing a remarkable influence upon it. At any rate, it must be recognized that in this case also the multitude of seed do not germinate in the first spring and that the germination percent in the second spring is higher than in the first spring.

A fact of some interest observed in the course of the above experiment is that the germination percentage in the first spring is higher in the seeds of smaller dimension. If we compare the size of the seed (represented by the average height of seeds in each fruit) in relation with the percent of germinations reckoned for each individual fruit, we can see that such small seeds as of fruits no. 722 and

TABLE 3. Delayed germination of the *Euryale* seed.

Material, seeds of autumn of 1927, sown directly after the harvest. Conditions in the germinator are the same as the group B in the preceding experiment except that the seeds were not sown directly in the mud, but were mixed with mud and then put into ERLENMEYER flasks, which were sunk into the bottom of the germinator.

Lot	Average Height of seed in each fruit (mm.)	no. seeds tested Oct., 1927–May, 1928	Number of seeds sprouted			g % * for 1928	Number of seeds sprouted					g % * for 1929
			June 1928	July 1928	Aug. 1928–Dec.		Jan.–Apr. 1929	May 1929	June 1929	July, 1929	Aug. 1929	
no. 721	11 68	17	7	3		21.3		1	10	0	0	23.8
no. 722	9 02	61	15	7		34 2		14	11	0	0	39.1
no. 723	11 18	33	5	0		15.2		7	14	0	0	63.6
no. 724	9 98	91	0	2		2.1		11	13	0	0	25.5
no. 726	10.48	74	16	1		23.0		0	11	0	2	17.6
no. 727	13 72	32	4	0		12 5		6	10	0	0	50.0
no. 728	10.86	56	2	8	no germination	17.9	no germination	0	7	2	1	17.9
no. 729	9.96	76	9	2		14.5		9	8	0	0	22.2
no. 730	8.84	91	40	11		54.3		3	4	4	0	11.7
no. 731	14 16	22	0	1		4 5		0	8	0	0	36.4
no. 732	14 71	22	4	0		18.1		9	6	0	0	68.2
no. 733	11 65	44	0	1		2 3		0	0	0	0	0
no. 734	13.52	35	0	3		8.6		0	0	1	0	2.9
no. 737	10 56	54	5	0		9 3		5	10	0	0	27.8
no. 739	10.78	45	9	1		22.2		4	6	0	3	28.9
Total		792	116	40		19.7		69	118	7	6	25.3

* germination percent

no. 730 exhibit the highest value in the germination percent. Another proof for the similar fact is shown in Table 4, which is obtained by measurement with the same 156 germinated seeds in Table 3.

TABLE 4. Correlation between the size and the germination.

Height in mm	7	9	11	13	15	17	Total
Number of seeds	63	46	36	11	0		156

Although a few of the larger sized seeds do germinate in the first spring, the tendency is by far the more accentuated in the smaller

ones. If we take into consideration these properties in combination with the fact that the *Euryale* seeds from other origins than Zyûnityô-gata are as a general rule much reduced in size, their germination capability in the first spring may have some explanation.

The relation between the size of seeds and the germination capability in the first spring is proved also in the following table (Table 5).

TABLE 5. Correlation between the size and the germination.

Material, no 7 Sown on Nov. 22, 1927 Germinator condition is identical to that of group B in Table 3

Height of seeds	Number of seeds tested	Germination in the spring of 1928
over 10 mm	162	3 (1.9%)
under 10 mm	73	3 (4.1%)
Total	235	6 (2.5%)

In this case, the total germination percent is very low as compared with that of Table 4. At any rate, the germination of the smaller seeds is higher than that of larger ones.

5 EFFECT OF SOME EXTERNAL FACTORS ON THE GERMINATION.

a. Temperature relation.

The relation of temperature to the seeds of *Euryale* is not always the same; seeds in different stages of the aging process behave themselves in different way with the same temperature.

We have already demonstrated that the seeds freshly harvested can not be induced to precocious germination by raising the temperature.

Seeds in a somewhat more advanced stage but not yet fully after-ripened sometimes react with positive result on application of high temperature. The effect is, however, far from remarkable and the germination percent never attains to a high value at the best. An example of such a case is shown in the following experiment. *Euryale* seeds of the lot no. 6, being kept in the laboratory room under water (*Euryale* seeds can not be stored long alive in dry state. The necessity of wet storage is imperative to keep their viability. In the present paper, readers are requested to understand that all the seeds employed

in the study were kept in water up to the time of experiment) since
the harvest, and were subjected to study on Jan. 7, 1927, i.e., after
some three months of storage. They were put in ERLENMEYER flasks
filled with tap water, and incubated at four different temperatures, in
the dark. The germination percent after 45 days is shown in Table 6.

For seeds in such stage, 15° was proved to be subminimal and no
germination occurred at this point. At 20° and 25°, higher germination
percent was attained and at 30° the value falls again. As a whole
we cannot obtain a high rate of germination at all.

In contrast to this result, seeds of *Euryale* which have probably
advanced far in the course of after-ripening, not only effect germination
already at a low temperature, but also react quite active when incubated
at a higher temperature. We collected in April of 1928 a number
of *Euryale* seeds from the sedimental mud at the water bottom of
Zyûnityôgata swamp, where the seeds themselves had been laid un-
disturbed since the separation from the mother fruits (We cannot tell

TABLE 6.   Germination at different temperatures.

Temperature	15°	20°	25°	30°
Number of seeds tested	33	25	55	27
Number of seeds sprouted	0	8	21	2
Germination percent	0%	32.0%	38.2%	7.4%

the exact age of each seed. The lot probably consisted mostly of
the seeds of 1927 and 1926. Older seeds are, however, not to be
discriminated). Some seeds of this lot sprouted already in the course
of transportation from their locality to our laboratory, regardless of
the precaution of keeping them always under 15° by means of a
DEWER's vessel. The germination percent reached as high as 28.2%
in three days in the course of transportation (vide supra, p. 57). With
the remaining seeds keeping immobile up to the moment, the study
with respect to the temperature effect was carried out. The condition
of experiment is identical in general to that of Table 6. The study
was commenced on April 21, 1928. Owing to the shortage in the
quantity of the material, only two degrees of temperature were tested.

The results are shown in Table 7.

As was stated above, this lot of seeds employed had already finished some germination previous to the commencement of this study.

TABLE 7.   Germination at different temperatures.

Temperature	20°	25°
Number of seeds tested	31	60
Number of seeds sprouted	23	48
Germination percent	74.2%	80.0%

So that the real germination percent must be still higher than the apparent estimate in this table which is calculated from the actual count in the present experiment. Taking these circumstances into consideration, we can conceive the fact that after-ripened seeds of Euryale are by far the more ready to germinate than the seeds not after-ripened. The cardinal points were not to be determined from the insufficiency of the material, yet the result tells the superiority of 25° to 20° in favoring the germination, a parallel phenomenon to the result in Table 6.

b.   Light relation.

There are reported a number of plant species whose seeds are very exacting with respect to the light requirement. On the one hand, many seeds can germinate only in the presence of the light (or prefer by far the light to the dark), while some others, on the contrary, are germination incapable or suffer from serious inhibitory influence under illuminated condition. Viscum album (WIESNER, 1897, p. 506), some Gesneriaceae (FIGDOR, 1907), Ranunculus scleratus (LEHMANN, 1909) and Chloris ciliata (GASSNER, 1910), etc. represent examples of the former category. As for the latter, a rather smaller number of cases are established in comparison to the former. Such are, for example, Phacelia tanacetifolia (REMER, 1904), Acanthostachys strobilacea (HEINLICHER, 1903), Veronica Tournefortii (LEHMANN, 1919), Nigella sativa, and N. damascena (KINZEL, 1908). Apart from these specialists, however, a multitude of seeds are indifferent and can

germinate indiscrimately in either the presence or absence of light.

The seeds of *Euryale* also seem to belong to the last category. The notion at least that their delayed germination is not conditioned by the light relation is supported by the result of repeated experiments that the seeds freshly harvested are susceptible to forcing neither in the light nor in the dark with all the best combination possible of other germinative conditions. As for the seeds fully after-ripened, no special experiment concerning the light requirement has been conducted up to this day. Observation at the natural habitat, however, suggests their indifference to the light. For the seeds in the natural habitat are observed to germinate either on the surface of the mud or buried deep. In the laboratory also, though they were tested in the dark as a general rule, germination in the light was not infrequently encountered by chance. All these observations indicate at least that they can germinate either in light or in the dark if they are sufficiently after-ripened.

c. Oxygen relation

Oxygen supply is a matter of importance among the environmental factors influencing the germination, seeds of most plants being incapable of germination when the oxygen supply is reduced (SCHEIBLE, 1900) or eliminated (HARTLEB and STUTZER, 1897, KRAUS, 1901). For some other species, the absence is said to effect even the total loss of viability (MAZÉ 1900). It cannot, however, be overlooked that seeds of some plants are germination capable with a meager supply of oxygen. Such is reported of some land plants (GODLEWSKI, 1904; LEHMANN, 1912), but is observed much oftner with aquatic ones (TAKAHASHI, 1905, with *Oryza sativa ;* MORINAGA, 1926 with various plants; TERA-SAWA, 1927, with *Trapa*). It is reported by MORINAGA (1926) further-more that the reduction of oxygen supply exercises in some cases with aquatic species even a better influence on the germination.

In order to obtain some knowledge with respect to the oxygen requirement of *Euryale* seeds I carried out the following experiment. Experiment 1. Material, lot no. 735. Duration of experiment, 2/II to 1928 1/III. Temperature, 25°. Light, excluded. Medium, 0.1% KNOP's solution, about 100 cc. was allowed per 10 seeds. A. in full oxygen pressure: the flask containing the seeds was set in a NOVY

jar into which pure oxygen gas (supplied from the market) was passed
for about one hour. The gas was renewed every week. B. in
anaerobic condition : the medium was boiled directly beforehand, quickly
cooled to 25°, filled in the flask to the brim, added with the seeds
and then promptly stoppered with a rubber stopper. The latter is
bored through with a glass tube which is conducted into mercury in
a test tube. This arrangement proved effective in keeping the flask
from breaking through the change in the hydraulic pressure of the
content, caused by chance variation of the temperature. C. control :
the flask was kept unstoppered in the ordinary air of the incubator.

Result of experiment is shown in Table 8.

TABLE 8.   Effect of oxygen on the germination.

Condi- tion	Number of seeds tested	Germination in				Final germination percent
		1st week	2nd week	3rd week	4th week	
A	20	8	2	0	0	50%
B	20	8	4	1	0	65%
C	20	6	5	1	0	60%

Experiment 2. Material, lot no. 8. Duration of the experiment,
21/IV to 18/VI, 1928. Temperature, 25°. Light, excluded. Medium,
water of garden pond, about 100 cc. was allowed per 10 seeds. A,
B, C denote the same as in the preceding experiment. Result of the
experiment is shown in Table 9.

TABLE 9.   Effect of oxygen on the germination.

	Number of seeds tested	Germination in				Final germination percent
		1st. week	2nd. week	3rd. week	4th. week	
A	27	5	7	0	0	44.4%
B	25	13	2	0	0	60.0%
C	60	37	11	0	0	80.0%

From the experimental data above tabulated we get to the con-
clusion : —

1. The seeds of *Euryale* are capable of germination under anaerobic condition.

2. Even when the atmosphere is entirely replaced by pure oxygen, they show quite a high percentage of germination.

3. Among the three conditions tested, viz., ordinary atmosphere, oxygen free, and full oxygen pressure, the first two were proved to be almost equally fitted to induce the germination, the last being a little inferior.

4. These facts indicate that the act of germination of *Euryale* seeds, at least in the early stage proceeds rather indifferent to the oxygen supply.

The reader may further refer to the section on the experiment to bring up artificially the isolated embryo (Section 10, d), where the oxygen relation is dealt with again to corroborate the view given here.

6 WATER RELATION.

The water content of fully ripe seeds of an ordinary land plant is known as a general rule to be about 10 percent (SKENE, 1924, p. 399). Therefore, when transfered to germinative condition, they have first to imbibe much water through the seed coat in order to activate the intracellular process to commence the germination.

Therefore, if this preparatory procedure of water imbibition is inhibited from some cause or other, the seeds cannot but remain immobile in their incasing structures. The most usually observed cause of this phenomenon is the impermeability of the seed coat to the water. The seeds of which the dormancy is ascribed to their coat of such obstinate property are known by the name 'hard coated seeds' or simply 'hard seeds'. Examples of such are not infrequently encountered in nature, and the *Leguminosae* are especially noted in this respect. Observations and experiments concerning the mechanism of this phenomenon and the methods to overcome it were treated by many investigators (see the review on pp. 48–49).

The question if the delayed germination of *Euryale* seeds are also caused by similar condition may certainly be answered in the negative, considering the invalidity of decortication to force the seeds, which fact is treated in the latter section (Section 8.).

Apart from this point, however, the water relation of seeds cannot
be neglected in the physiology of germination, and it may be worth
while to report some experimental results along this line.

a. Water content of the seeds.

The water content of the *Euryale* seeds tend to be reduced towards
the time of maturation. The actual course of the change, however,
is not yet traced in detail. As for the fully matured seeds which are
ready to be delivered freely from the degenerating mother fruit, the
dry matter is usually found to be a little above 50 percent. In a
case with the sample of 1928, I have once obtained a result of 55.2
percent dry matter (mean value of ten seeds from one and the same
mother fruit. The seeds were 12.0–12 2 mm high), but such high
value seems to be of a rare occurrence, and dry matter content of
only a little over 50 percent is more usual.

Distribution of the water in the parts of the seed is not uniform.
Calculation from the data in Table 1 (in the appendix) gives the
following values as the dry matter contents of the parts of a seed.

Whole seed	51.0 ±1.354%
Seed coat	44.13±1.701%
Inner part	59.18±1.064%

It is shown that the water content is a little less than half in the
whole seed, higher in the seed coat, and much less in the inner part.
At any rate, if compared with the land plant, it will be realized that
the *Euryale* seed is quite rich in water.

We have next studied whether seeds of such a high grade of
water content are actually saturated to the full, or whether they are
capable of further imbibition of water in the case of elimination of
inhibitory factors if any. The first method thereto is to decorticate
a seed, counterpoise, put into water of suitable temperature and note
the change in weight by repeated weighing at interval. During the
act of weighing, the decorticated samples were kept in a small weighing
bottle with a little water to keep them from loss of moisture through
evaporation, and we could thus reduce the error of measurement less
than 0.002 g. Tables 10 and 11 are the results of these studies.

TABLE 10. Change in weights of decorticated seeds in water.

Material, lot no 66   Temperature, 25°   Medium, tap water

	Jan. 27 (1927) 18 h	Jan 28 12 h	Jan 29 16 h
a	0.6042	0.6042	0.6041
b	0 6155	0.6155	0.6148
c	0.4905	0.4985	0.4983
d	0.6600	0.6600	0.6601
e	0.6740	0.6751	0 6749

TABLE 11. Change in weights of decorticated seeds in different media and at different temperatures.

Material, lot no 66.

	Temp	Medium	Weight (mg)		
			9/11 '27 16 h	10/11 15 h.	11/11 17 h.
a	25°	Tap water	674.0	670.4	667.4
b	,,	,,	492.7	491.3	490.4
c	Room temp	0.05% Knop's solution	582.6	580.5	578.9
d	,,	,,	519.3	517.0	516.6
e	,,	Tap water	601.8	601.4	600.8
f	,,	,,	568.3	564.1	563.8

It may be concluded from these results that the seeds of *Euryale* are always saturated with water and the elimination of the seed coat has no effect to force the seed to absorb more water. In some cases even reduction of weight, though rather insignificant, was observed. This may be explained by the diffusion of the matter away from the seed thanks to the elimination of the seed coat. Exact study about this matter was not carried out further.

The above expressed opinion that the seeds need no more water in the actual procedure of germination was also realized from the following experiment.

We selected for the material to study such a lot of seeds of which we were sure of quite a high percentage of germination by preliminary

study. A number of such seeds were each weighed, laid in germinative condition and a daily record of change in weight was kept until the commencement of germination. Example of record with a few seeds is shown in Table 12.

TABLE 12.   Change in weight of whole seeds.

Material, lot no 61   Date of exp, 3/II, 1927   Medium, tap water.
* indicates the commencement of germination on that day.

Temperature		20°	20°	20°	30°
Weight (mg.) of seed, after	1 day	971.1	1065.7	1290.4	931.5
	2 days	969.9	1064.9	1288.4	931.4*
	7 „	972.9	1067.3	1292.1	
	10 „	973.0	1067.3	1292.2	
	17 „	971.5	1064.9	1292.2*	
	20 „	971.7*	1064.9		
	28 „		1065.1*		

We can hardly recognize any increase in weight beyond the range of experimental error, so that we may safely conclude that the preparatory imbibition of water is not required by the germinating seeds, or that the delayed germination of *Euryale* seeds has no concern with the water exclusion due to the seed coat.

Another support to this conclusion is furnished by the behavior

TABLE 13.   Change in size of embryo isolated from the seeds.

Material, lot no 740.   Date, 15/II, 1928.   The unit of measurement denotes the division of an ocular micrometer corresponding to 1/30 mm.

	Initial size		Final size	
	Length	Width	Length	Width
a	85	80	85	80
b	76	75	75	75
c	75	77	75	78
d	73	79	75	78
e	77	78	77	79
average	77.2	77.8	77.4	78.0

of the embryo itself when it is isolated away from the seed. In such case, the embryo is not observed to be favored in absorbing water. It remains the same after the manipulation as before, or it appears indifferent whether the free supply of water is permitted or not.

In Table 13 are recorded the magnitudes of embryos at the moment of isolation and after a period of subsequent incubation at 25° in tap water for 48 hours.

The matter treated in this section may be briefly summarized : —

1. *Euryale* seeds are possessed of a comparatively larger amount of water from the outset.

2. The inner parts, viz., embryo, endosperm and perisperm are fully saturated of water and we can induce no further imbibition by means of decortication.

3. Preparatory water intake does not seem necessary for germination.

4. The delayed germination cannot be ascribed to the water exclusion, if any, by the seed coat.

b. Water permeability of the seed coat.

From the fact just described in the preceding section that the seeds of *Euryale* are saturated with water, we can naturally presume that the seed coat must not be impermeable to water. Of course, the water passing through the coat must encounter a certain degree of resistance and the velocity must be more or less diminished in comparison with the case of free passage. In connection with the problem in the preceding section, we planned some experiment to determine the relative resistance of the seed coat to the passage of water. The procedure of the experiment is as follows.

The seed is first bisected along the equatorial plane, and scraped away of its inner portion to obtain from one seed two hollow hemispheres of coat. The test material thus prepared is mounted, along its circular rim, on one end (upper) of a small piece of glass tube (A) of the same width as that of the seed. Venetian turpentine is used for cementing. The other (lower) end of the tube (A) is connected to the lower end of another tube (B) with a rubber tubing. After the set is completed, it is filled with water previously boiled

and rapidly cooled. Both tubes are fixed in vertical position and the free water surface in the tube (B) is covered with a thin layer of olive oil to prevent evaporation, and is arranged so as to keep the same level as the upper end of the tube A.

In the course of time, the water passes away through the surface of the seed coat and the evaporated volume may be calculated from the depression of the niveau in (B). As a check test, the evaporation through a gypsum cast of the same cast as the material was employed. In Table 14 is shown an example of experimental results. The resistance recorded there denotes the reciprocal of the quantity evaporated in 24 hours.

TABLE 14   Evaporation through the seed coat.

Size of the seed 64 a, 13 mm high and 12 2 mm wide   63 b, 13 mm high and 12 0 mm wide Thickness of the coat, 1 mm   Temperature, 11°-20°. Relative humidity, 82–86 Date of experiment, 14–15/III, 1927. microp., micropylar hemisphere. antim., antimicropylar hemisphere.

Lot	Part	Evaporation in 24 hours (cubic mm)	Resistance	Ratio of resistance
no 63 a	microp	60 84	0.01643	
,,	anti-m	59.46	0.01682	16.95
,,	total	120.30	0.008312	
no. 63 b	microp	42.59	0.02348	
,,	anti-m	42.59	0.02348	
,,	total	85.18	0.01174	
average	,,	102.74	0.009740	19.64
control	hemisph	1008.0	0.0009922	1.00
,,	2 hemisph.	2016.0	0.0004961	1.00

It is demonstrated that the seed coat of *Euryale* is permeable to water although the resistance is quite high in comparison with the same thickness of gypsum.

We have described already in the former section the structure of the seed coat and shown that it is not of uniform constitution. Then it is quite natural to expect that the distribution of the above said resistance among the different layers of the coat is not uniform. So that is was intended in the present study to determine the relative

degree of resistance of each different layers. As a matter of fact, however, it proved impracticable to test each separate layer one by one, for some of them were devoid of sufficient mechanical independence owing to their thin construction. These circumstances compelled me to limit the comparison to one case, viz., the outer two layers combined versus the inner two also combined. Between these two combinations, there exists a plane of weakness, which is ready to separate the two if only a slight strain is applied.

For these reasons, only the comparison of the outer and the inner part of the coat was studied. An example of the results is shown below in Table 15, in which was employed the one of the materials already tested in the above table, viz., the antimicropylar half of no. 63 a. This material, directly after the close of the former experiment, was separated into two parts, i. e., the outer two layers and the inner two, and the water permeability was determined for each, by the same method as described before.

TABLE 15. Comparison of the water permeability of the different layers of the seed coat.

Temperature, 12°-20°. Relative humidity, 82–86. Date of experiment, 16-17/III, 1927. Control is the same as in the preceding table

Part of the seed coat	Relative thickness	Evaporation in 24 hours (cubic mm)	Resistance	Ratio of resistance	
Outer layers	66	340 7	0.0029	18.5	
Inner layers	7	77.29	0.0129	81.5	
total			0.0158	100.0	17.1
control	73	1082.	0.0009241		1

The separate layers exercise by far the smaller resistance to the passage of water than the whole layers combined, but the resultant sum of each separate resistance approximates the single resistance of the whole layers combined. As for the relative resistance of the parts of the coat, there is demonstrated a remarkable contrast between the outer and inner layers. The latter, notwithstanding its minor thickness, proved to be very resistant to the passage of water. This characteristic is probably due to the suberized cell wall in the innermost layer.

7 MECHANICAL RELATION.

Of the functions of the seed coat, one of the most fundamental significance is the protection of the inner structure from injurious influences from outside. The resistance to the mechanical interferences is in particular aimed at in many seeds, and cases are not infrequently encountered where the character is accentuated far beyond the practical necessity. In such cases, not only the external stress is antagonized, but also the enlarging of the embryo itself is much interfered with, until at last in some extreme cases the imbibition of water by the embryo is almost wholy prevented, thus causing apparent dormancy of the seed. Examples of such are shown by CROCKER (1907) and by CROCKER and DAVIS (1914).

As for seeds of *Euryale*, it is demonstrated in the preceding section that the seeds are well saturated with water from the outset. The inner part of the seed has, so far as the imbibition of the water is concerned, no relation with the mechanical resistance, if any, exercised by the seed coat. If, however, once the act of germination takes place and the cells of the embryo grow, the latter has first to overcome the mechanical interferences of the seed coat in order to develop away from inside of the incasing structure. In this state of the matter, the problem belongs to the physiology of a seed already commenced germination or a seedling, and is no more within the reach of the phenomenon of the dormancy, the principal theme of the present study. However, from the general viewpoint of the germination physiology, the matter is of no little consequence and it may be worth while to treat of the experimental results along this line.

a. The relation of the embryo and the seed coat in the course of the germination.

The seed coat consists for the most part of a thick layer of highly developed sclerenchymatous cells and constitutes so resistant a structure that it proves very hard to destroy it. But, so far as the act of germination is concerned, this great resistance of the whole seed coat exercises almost no effect except at a limited region, that is to say, at the micropylar operculum. If once the act of germination takes

place, this latter structure has to be replaced out of its position by
the elongating hypocotyl. Through a minute perforation thus originated,
the cotyledonal stalks of the embryo elongate themselves, and the
young plant comes into sight pari passu. (OKADA, 1925). The embryo
needs to overcome only the resistance of the operculum which is keep-
ing its position with the surrounding part. The necessary mechanical
work is naturally limited to a small region. Moreover, the suture line
of the operculum with its surrounding part cannot be very solid, for,
as is plainly shown in former section (Section 3), the epidermal layer
is much reduced in its thickness along
the suture line, and the cell wall of the
underlying layer is thin and non-lignified
(p 53 and Text-fig. 1). The embryo
concentrates its force of growth against
this weak point, leaving the remaining
part of the seed coat undisturbed.

Text-fig. 4   Apparatus to
test the resistance of the oper-
culum. g. gypsum block, o,
operculum; w, weight.

b.   Resistance of the operculum.

The measurement was conducted in
the following manner.

First the seed to be tested is bisected
along the equatorial plane by means of
a small saw, and from inside of the
micropylar hemisphere, the inner struc-
tures were scraped away, taking precau-
tions not to disturb the connection
between the operculum and the sur-
rounding part. This hemisphere is sub-
jected to the test. In the meantime, a
small gypsum block was prepared with
a depression on the upper surface to fit
the above hemisphere. Another smaller
depression is made further in the first
one at the bottom. Now the test material
fitted in the first depression in the gypsum
block is brought under a small vertical

shaft which is freely movable up and down. The lower tip of the
shaft is adjusted to press just against the operculum and subsequently
weight is charged little by little (by 0.5 g) to the shaft until the
operculum yields to the charged pressure and falls into the smaller
depression. The general arrangement of the contrivance is diagram-
matized in Text-fig. 4. The weight of the shaft plus the charge at
the critical moment was accounted for the resistance of the operculum,
and from the area of the latter, the value was changed into atmospheric
pressure by calculation. The tests were made either in wet or in
dry state. Results are shown in Table 16.

TABLE 16. Resistance of the operculum.

Condition	Pressure applied (in g.)	Mean diameter of operculum (in mm.)	Pressure (in atm.)
	28.5	1.5	1.56
Wet	61.5	1.6	2.96
	35.5	1.6	1.71
average			2.07
	136	1.6	6.55
Dry	52.5	1.5	2.87
	56.0	1.5	3.07
average			4.16

The obtained results are rather variable, but the general tendency
can be perceived that the resistance is by far the smaller in the wet
condition than in dry, a fact demonstrated by MÜLLER (1914) with
many seeds. As the seeds of *Euryale* germinate ordinarily under
water, it needs no special mention that the resistance in the real case
is limited to that in the wet condition.

c. Force of growth.

Measurement was performed by means of a small dynamometer
after MÜLLER (1914). A slight modification was made to the apparatus
in the fixing device of the seed. As the incasing structure of the
Euryale seed is highly resistant and the growing embryo has no
concern with it except at the operculum, the seed under test was fixed
in the proper position by directly pressing it between two horizontally

movable platelet. The seed was arranged in such a position that the
axis of the embryo comes vertically under the spring of the apparatus.
The measure spring was of such a strength that the approach of the
indices is 1.9 mm. per 100 g. of the charged weight. During the
course of the measurement, the lower part, of the apparatus was
dipped in water up to the level of the top of the seed, so that the
embryo was kept moist always.

     The procedure of the actual measurement is as follows: — A
number of after-ripened seeds were incubated in a proper germinative
condition and kept under incessant observation. If any seed of them
were found to indicate a first sign of germination (the beginning
dislocation of the operculum), it was taken immediately, fixed in the
proper position on the dynamometer, gently pressed down with the
lower plate of the spring against the micropylar pole of the seed
(the initial pressure to be applied is some 150 g.), and then allowed
to grow at a constant temperature of 25°. After a lapse of some
time, the embryo is sure to grow, push against the compression of
the spring and the indices naturally approach themselves, until at last
the force of growth of embryo attains its maximum and the indices
remain stationary.   Then the approached distance is measured by
means of a horizontal microscope. The value obtained is changed
into the pressure and, further, from the cross sectional area of the
embryo, the value is changed again into atmospheric pressure.   Thus
the force of growth of the embryo charged on the operculum is
estimated. The results of the measurement are shown in Table 17.

TABLE 17.   The force of the growth of the embryo.

Lot	Force (g.)	Mean diameter of the cross section of the embryo (mm)	Force (atm.)
no. 739	374	2.7	6.32
no 8	311	2.6	5.67
no 8	225	2.34	5.06
no 8	345	2.63	5.76
no. 8	265	2.57	4.76
average	—	—	5.51

Thus we estimate the force of growth of the germinating embryo

roughly to be 5 atm., by far stronger than the resistance of the seed coat in wet state. Therefore, if the act of germination is once commenced, the operculum yields readily to the pressure from the part of the embryo and the latter finds its way to the external world through the perforation thus originated.

8. EFFECT OF DECORTICATION.

According to the generally accepted conception on the problem of delayed germination, the hindrances in the part of the seed coat are ascribed either to the mechanical interference of the seed coat in inhibiting the inner part from free imbibition of water or to the impermeability of water or oxygen through the seed coat.

As for the seeds of *Euryale*, the view that neither of these conditions seem to be applicable is easily conceivable from the data in the foregoing sections. Thus the expectation naturally follows that the elimination of the seed coat may produce no effect in awakening the dormant seeds. This view is already proved in my previous work (OKADA, 1925). The experiment below is another example to show the inefficacy of decortication.

Experiment : — On 20/II, 1926, 30 seeds of the lot no. 5 were desinfected by dipping in 1 : 1000 solution of corrosive sublimate for ten minutes, and decorticated with sterile knife (the whole part of A and B layers with the perimicropylar part of C layer were scraped away). They were then each put into a test tube. For the germinative media, ordinary tap water was given for ten of the seeds and 0.1 % KNOP's solution for the remaining twenty. The vessels with the media were previously autoclaved. Incubation at 25° in the dark. After the lapse of a month no symptom of germination was observed. The culture was kept further for one year, in the latter part of which period all of the tubes were infected with microorganism. Some of the seeds appeared still quite healthy notwithstanding the contamination, but none of them ever germinated.

9. EMBRYONIC CHARACTERS.

We have dealt in the preceding several sections with the study of the seed coat characters in connection with the problem of dormancy,

to arrive at the conclusion that there are no direct relations to be accepted. Then it follows naturally that the dormancy must be due to some other part than the seed coat. Now, of these remaining parts of the seed, the embryo itself is first worthy of study as it essentially represents the future life. In the study of the embryo, again, two cases must be distinguished, the one of morphological, and the other of chemical significance. The former case is observed in such seeds as are provided with rudimentary embryos which are not sufficiently differentiated. For such incomplete embryos, the necessity of after-ripening is almost imperative, during which period, they grow on and accomplish differentiation, until at last they assume a certain definite structure and magnitude to commence germination.

Another possible case is presented by those seeds in which the embryos are, though quite completed morphologically, not yet sufficiently matured chemically, that is, the intracellular condition is not yet so arranged as to respond readily to a germinative condition. After a lapse of time, during which the necessary chemical changes are completed in the cells, the embryos become, for the first time, germination capable.

In order that we may determine to which of the two possibilities the case of the *Euryale* seed is due, inspection must be made into both the morphology and chemistry of the embryo. We will now proceed along this line of the problem.

a. Morphological.

The embryo of the *Euryale* seed is a rather minute body situated at the micropylar pole of the seed (Text-fig. 1, p. 52). The general appearance may be roughly described as globular. It is already differentiated quite well at the time of the harvest, and we can easily distinguish the plumule. cotyledons and hypocotyl. On the plumule, we can further discern 2-3 primordial leaves and in the cotyledons the rudiment of the conductive tissue. The hypocotyl is poorly developed, being represented by the connecting region of the cotyledons in pair.

As compared with these configuration of a fresh embryo, we will study next that of the embryo of an after-ripened seed. As the

material of the study, we have selected the lot no. 8, which had been exposed to after-ripening in the natural habitat and whose germination capability had been previously established by experiment. (Germination percent of at least 80%). 50 seeds of this lot were dissected, the embryos were examined, and the result proved that the multitude of them are not to be distinguished in their appearance from that of the fresh seeds. Nor was any improvement recognized in the differentiation of the structure. However, there were found a few seeds (8 out of 50) of which the cotyledons were swollen larger as compared with those of the normal ones. The possibility that such embryos are also capable of germination is supported by the fact that we find both normal sized and swollen cotyledonated embryos in case of examination of seeds just commencing germination. The rate of occurrence of the latter ones was 8 out of 50 in one case, and in an extreme individual, a cotyledon as long as 4.1 mm. (exclusive of the after germination increase) was measured. If the record in Table I in the appendix is consulted, we learn that the average length of cotyledon (practically the same as that of the embryo) in fresh seed is 2.75 mm., so that we can recognize a distinct enlargement in this example. In such enlarged cotyledons, the consistency seems also to be transformed and they appear decidedly more transluscent. As for the differentiation of the parts, no remarkable change was recognized.

Taking into consideration together these observed data, we may give the suggestion that the preliminary growth of embryo in the seed in the course of the after-ripening seems to be of rather rare occurrence, and in the multitude of cases such preparatory procedure can be totally dispensed with. The capability of germination of embryos with cotyledon enlarged previously is also not to be excluded.

b. Chemical.

Composition of the embryo of the *Euryale* seed was studied provisionally by means of the microchemical method. Results of studies with the embryo of freshly harvested seed are tabulated below (Table 18). For convenience sake, data concerning the perisperm and endosperm are also annexed.

TABLE 18. Microchemistry of embryo, endosperm
and perisperm.

Object of study	Part of seed			Method of test
	Embryo	Endosp	Perisp.	
Starch	—	—	++	Staining with I-IK-solution
Reducing sugar *	—	—	—	Reduction of FEHLING solution
Fat	++	++	—	Staining with Sudan III
„	—	—	—	Reduction of osmic acid
Protein	++	++	+	MILLON's reagers
„	+	+	+	Biuret reaction
„	+	+	+	Xanthoprotein reaction
Catalase	++	++	+	O_2-evolution from H_2O_2
Oxidase	—	—	—	Oxidation of guajac tinct
Peroxidase	—	—	—	Do. in the presence of H_2O_2

++ positive reaction remarkable, + positive reaction recognized, — negative result.

As for the composition of the after-ripened seeds, similar studies
were carried out. Of those substances tested, we could recognize no
remarkable difference so far with starch, fat and protein by means of
the microchemical method. The case was dissimilar with reducing
sugar. In this latter substance, the distinction was found to be con-
siderable. While we could demonstrate no sugar in fresh seeds, it
was encountered with a multitude of after-ripened seeds which react
positive to the sugar test. The presence of reducing sugar is shown
most clearly in the embryo and in the endosperm, but we can demon-
strate it also in the perisperm region. Hence it may not be very
improbable that the sugar is produced through the hydrolytic decom-
position of the starch ** in the perisperm, and then transferred to the
embryo. According to this suggestion, the antecedent action of the

* In order to determine the kind of reducing sugar, the methods after SENFT (1904)
  and GRAFE (1905) were applied. The treated preparations produced in both
  cases a multitude of minute yellow particles, but any trace of the typically
  bundle shaped crystals was never discovered, so that exact d............. .
  not achieved. In the table, therefore, it is denoted simply as reducing sugar.
** The decrease of starch due to this cause seems to be quite insignificant in cc...
  parison with its total quantity, so far at least as the actual germination does not
  take place. That is why we could not confirm the change in quantity of starch
  during the after-ripening period simply by means of the microchemical method.

hydrolytic agent must be assumed. If such is really the case, the production or activation of amylase preceding the appearance of reducing sugar is to be expected. The positive result in the experiment to force the seed to germinate by the application of amylase (vide infra, p. 96) seems to support the probability of this view.

Further, if this suggestion be really the case, some other protoplasmic changes simultaneous to, or rather more probably preceding, the appearance of amylase activity are to be expected as well. The hitherto reported changes in the course of after-ripening of various plants, for instance, the activation of catalase (ECKERSON, 1923, p. 290, with Crataegus*; SHERMAN, 1921, pp. 7-8, with Crataegus; ROSE, 1919, p. 293, with Tilia; CROCKER and HARRINGTON, 1918, pp. 158 9, with peach; JONES, 1920, pp. 141-3, with sugar maple; DAVIS, 1927, pp. 239, 255, with Cornus and Sambucus), the increase in acidity and titrable acid (ECKERSON, 1913, pp. 290-291, with Crataegus; ATWOOD, 1914, pp. 407-8, with Avena fatua; ROSE, 1919, pp. 290-2, with Tilia; JONES, 1920, p. 146, with sugar maple; PACK, 1921, 1, pp. 146-7, with Juniperus; DAVIS, 1927, pp. 245-6, with Cornus florida **) etc., may be considered to represent some phases in the connected mechanism. Study along this line of the problem is also in progress with the present material, the result of which I hope to report in the very near future.

The parallelism between the appearance of reducing sugar and the improvement in the percentage of germination is reported with seeds of many plant species (ECKERSON, 1913, with Crataegus mollis; ROSE, 1919, with Tilia americana; JONES, 1920, with sugar maple; PACK, 1921, with Juniperus; JONES, 1923, with Ilex opaca; DAVIS, 1927, p. 246, with Cornus florida). It is explained by the facility in the transformation of the energy offered in the form of reducing sugar, thus allowing supply of easily utilizable energy to commence the act of germination. The close relationship of the two phenomena is further proved by the experiment in a later section (pp. 86-88) where is demonstrated the efficacy of forcing isolated embryos by the

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* Slight increase was noticed by microchemcial method.
** The increase in amino acid was proved macrochemically. As for the hydrogen ion concentration of the seed of this plant, any remarkable change was detected by neither the colorimetric nor the quinhydrone potentiometer method.

artificial supply of sugar from outside.

It must be noticed, however, that the presence of sugar is not an imperatively indispensable condition for the commencement of the germination. For we have encountered at times such seeds just beginning germination as react negative to the sugar test (in the stage when the operculum is being pushed away by the elongating embryo). Such examples were found to occur more in seeds forced by high temperature. The fact suggests that other condition or combination of conditions may as well substitute for the function of sugar and effect the mobilization of the embryo.

As for oxidase and peroxidase, the former seems to appear in the embryo and endosperm, but not in the perisperm of sufficiently aged seeds, while the latter cannot be detected clearly in any of these parts. A more detailed study, however, is to be accomplished with respect to these ferments.

Form the data so far mentioned, we may conclude that the chemical properties of the seed suffer as a general rule some transformation. As other possibilities which are reported to be responsible for causing delayed germination do not seem to fit the present case, this last theory must be adopted, that is, the chemical nature of the seed is so transformed in the course of after-ripening as to become easily susceptible to the germinative stimulus of the environmental factors. The appearance of sugar represents one phase of such transformations.

10 CULTURE OF ISOLATED EMBRYOS

Through the preceding sections we have engaged in the study of the delayed germination of *Euryale* seeds to attain the conclusion that their dormancy is attributable to the properties of the embryo itself. We have learned further that the cause of the dormancy is not of morphological but of chemical character, that is, so far as the structure is concerned, the embryos of *Euryale* seeds are sufficiently matured when the fruits separate from their mother plants, and the necessary process during the period of the after-ripening is concerned almost exclusively with the chemical changes in the embryo. To what extent the latter changes concern or which aspect of them is

to be appreciated to be of the most fundamental significance, we know but little. There is much left to be pursued along this line of problem. However, if we limit ourselves to the knowledge already obtained, we may mention some points of not minor significance. We know, for instance, that the embryo shows a remarkable distinction at different stages with respect to the sugar content; the embryo of the seed freshly harvested contains no sugar in general, while we can demonstrate its presence in the multitude of samples after-ripened. Taking these circumstances into consideration, it may not be very inconsistent to assume that the appearance of sugar indicates at least a favorable condition to induce the germination. We have started some experiments with these assumptions as working bases, and have experimented to determine whether an embryo isolated from its surrounding attributes (seed coat, endosperm and perisperm) may be artificially forced to germinate by means of an external supply of sugar. The study may serve as a parallel proof to the statical study of the chemistry of the embryo, and furthermore, some of the problems in the germination physiology may as well be studied with the isolated embryo. With these expectations in view, experiments were carried on of which the results are arranged in the following lines.

a. Method.

The problem whether or not the isolated embryo is capable of growth by an artificial culture method outside the own seed has been studied by many investigators (see the review in detail in the paper by STINGLE. 1907), and it was achieved by some (HANNING, 1904; DIETERICH, 1924; YOSHII, 1925) to bring up the isolated embryos of even immature seeds of some plant species.

The seeds of *Euryale*, although they are apparently fully matured so far as the morphological characters are concerned, are none the less incapable of germination before the after-ripening. I have therefore started the study first in testing whether the embryos are ever capable of being forced in culture media recommended by former workers in bringing up immature embryos.

After the attainment of positive results in that experiment, I tried further to analyze the effect of the ingredients of the media, which

study proved the dominant importance of the contained sugar. The study
was then extended to compare the effect of various kinds of sugars and
also of different concentrations. Along with these studies, the oxygen
relation of the embryo in artificial culture and the different effects of
media due to their hydrogen ion concentrations were investigated.

Before proceeding to report on the experimental data, remarks
will be given concerning the general method of experiment applied
throughout these studies.

For the materials of these studies, seeds of *Euryale* were employed
which were collected in the autumns of 1926, 27 and 28, and since
kept in the laboratory room until the time of experiments. They
were first dipped in 0.1% solution of corrosive sublimate for about
five minutes, washed with sterile water, and then with absolute alcohol,
rapidly passed through the flame of a burner, and dissected with a
sterile knife, to isolate the embryo from the surrounding structure
under as sterile condition as possible. As for the culture vessel,
ordinary test tubes (some 1.6 cm. wide and 20 cm. long) were invariably
employed. Culture media were divided into these tubes by 10 cc., and
then sterilized in flowing steam. When sugar solutions of high acidity
were employed, the sterilization was exercised separately with the
sugar solution and the acid solution. Into each test tube was placed
a single embryo. Incubation at 25°, in the dark.

In order to keep the culture from contamination, the preparation
of the embryo was necessarily conducted with especial care, and
accordingly the work proved to be rather troublesome and complicated,
which circumstance compelled the study to be made with a limited
number of samples. To compensate to some degree the shortage in
the number, all the embryos employed were discriminated with their
respective lot number of mother fruits, and in case of comparative
study, discretion was exercised to match the materials from the one
and the same mother fruit, so that the error due to the individual
difference of the fruit might be reduced as far as possible.

In the tables below, the results of the studies on the germination
of the whole seeds are also attached for the purpose of comparison.
These whole seeds were tested in uniform manner for all of the ex-
periments; they were sown in tap water, incubated at 25° in the dark,
and the germination percent was counted on about the 45th day.

b. Germination capability of isolated embryo.

As is stated above, the study was first undertaken to determine if the isolated embryo of *Euryale* is susceptible to forcing by means of artificial culture media. The solution recommended by YOSHII (1925, p. 108) to bring up the immature embryo of *Pharbitis* was first tested. The composition of the media is : — 0.1 % KNOP's solution 100 cc., Saccharose 2.5 g. and Asparagin 0.05 g. The results are shown in Table 19, and some of the samples in this experiment are shown in Plate I, fig. 1.

TABLE 19.   Germination of isolated embryo.

Date of experiment, 14/VI, 1927

Lot	Number of seeds tested	Observation after						Germ. pct. of whole seeds
		20 days		45 days		60 days		
no. 62	6	++	2	++	3			15.7%
		+	2	+	1			
		—	2	—	1			
		cont	1	cont	1			
						no increase in the count, see the note below		
no. 68	10	++	3	++	3			—
		—	6	—	6			
		cont	1	cont.	1			
no. 69	9	+	6	++	5			0%
		—	3	+	2			
				—	2			
Total	25			posit.	14			
				negat	9			
				germ. pct. *	60.9%			

The symbols and abbreviations in the table are :— ++, leaf expanded; +, plumule elongated; +, embryo elongated, these three belong to positive results —, no sign of germination; cont., contaminated; posit., positive result; negat., negative result; germ. pct., germination percent. These symbols and abbreviations hold good also in other tables in this section.

* the contaminated samples are excluded from the calculation.

From this experiment we learn that the isolated embryos prepared from dormant seeds are susceptible to forcing provided that a proper medium is applied.

Those embryos marked wit ⧻ in the table continued to grow further, but all the rest showed no remarkable change, so that if the mere percent of germination is accounted, there is no improvement in 60 days count. Therefore, the counts in 45 days may be regarded to represent the highest value of germination percent obtainable.

c. Significance of sugar to force the embryo.

In the preceding experiment, we employed as culture medium the KNOP's solution with sugar and asparagin added, and demonstrated its efficacy to force the isolated embryos. Now, in order to proceed further to determine to which component of the above medium the effect is due, we tested with various combinations of the above three components. As a control test, the behavior of isolated embryo in pure water was also studied. The results are shown in Table 20. (With respect to Tables 20–27, actual counts in detail are given in the tables at the end of the paper.)

TABLE 20. Comparison of the effect of each component.

Date of experiment, 10/II, 1928. Material, seeds of the autumn of 1927

		Sugar containing				Sugar free			
		I	II	III	IV	V	VI	VII	Germ. pct. of whole seed
Composition of media	0.1% KNOP's solution	100 cc	100 cc	—	—	100 cc	100 cc	—	
	Aqua	—	—	100 cc	100 cc.	—	—	100 cc.	
	Asparagin	0.05 g	0	0	0.05 g.	0.05 g.	0	0	
	Saccharose	2.5 g.	2.5 g.	2.5 g.	2.5 g.	0	0	0	0
Germ. pct. after 45 days		88.9	95.0	84.2	80.0	0	0	0	

The dominant efficiency of sugar is decidedly shown. The table demonstrates clearly that even the simple solution of sugar is effective enough to force the embryo while any combination without sugar is entirely invalid. Some of the samples in this experiment are also shown in Plate I. fig. 2.

It must be noticed here that the act of germination itself and the further development in the later days must not be confused. In these

tables, only the former phenomenon is treated. If the later develop-
ment is accounted for, the combination of KNOP's solution with sugar
is by far more effective than the simple sugar solution. This fact is
indicated to some degree in Table XX at the end of the paper, and
a little more clearly in Plate I, fig. 2.

While studying the truth of the dominant effect of sugar to force

TABLE 21. Comparison of the concentration of saccharose.

Material, seeds of the autumn of 1927. Date of exp., 30/XI, 1927

		I	II	III	IV	Germ. pct of whole seed
Composition of media	0.1% KNOP's sol.	100 cc	,,	,,	,,	
	Asparagin	0.05 g	,,	,,	,,	
	Saccharose	0 g	2.5 g	5.0 g	7.5 g.	
Germ pct after 45 days		0	69.2	71.6	40	0

TABLE 22. Comparison of the concentration of glucose.

Material, seeds of autumn of 1927. Date of exp., 28/XII, 1927.

		I	II	III	IV	V	VI	Germ. pct of whole seed
Composition of media	0.1% KNOP's solution	100 cc	,,	,,	,,	,,	,,	
	Asparagin	0.05 g	,,	,,	,,	,,	,,	
	Glucose	0.05 g.	0.1 g	0.5 g.	1.0 g.	2.5 g	5.0 g.	
Germ. pct. after 45 days		0	0	58.5	58.3	66.6	16.6	0

TABLE 23. Effect of laevulose and maltose.

Material, seed of the autumn of 1927 Date of exp., 17/II, 1928

	I		II		Germ. pct. of whole seed
Composition of media	0.1% KNOP's solution 100 cc.		0.1% KNOP's solution 100 cc.		
	Asparagin	0.05 g.	Asparagin	0.05 g.	
	Laevulose	1 g.	Maltose	2.5 g.	
Germ. pct. after 45 days	5.1%		31.3%		0%

TABLE 24. Effect of saccharose, maltose and lactose.

Material. seeds of the autumn of 1928. Date of exp., 17/I, 1929.

Composition of media	2.5% Saccharose	2.5% Maltose	2.5% Lactose	Germ. pct. of whole seed
Germ. pct. after 45 days	89.2%	96.2%	88.0%	3.6%

the embryo, experiments were extended to learn further the effects of various kinds of sugar, viz., saccharose, glucose, laevulose, maltose and lactose. Of these five, the first two were tested for their effects with regard to the concentration. The experimental results are compiled in Tables 21–24.

Of these four experiments tabulated above, the first three were carried on with culture media which contain, besides the sugar to be tested, KNOP's solution and asparagin. In the last experiment, simple aqueous solutions of the sugar in question were used. But, taking into account the insignificance of the other ingredients in comparison with sugar, those results may be still available in comparing the effects of different kind of sugar.

Now, on inspection of these tables, we can perceive the general tendency that the disaccharides are almost always very effective. If we compare the effects of saccharose, maltose and lactose (Table 24), the final germination percents recorded in the table are almost equally high. Further inspection shows, however, that the maltose seems most favorable for the growth of the embryos, if the developmental behaviors in a little later stage are consulted. (See Table XXIV in the appendix, where the actual counts are given in detail.) We obtained of such embryos as developed so far as to expand their leaves (those marked with ⊪ in the table), 5 in maltose medium, 1 in saccharose, and none in lactose. These results indicate that the maltose is best fitted for the growth of the embryo.

Hexose seems to be less effective. Although the value in Table 22 is quite high, the later development of the embryo is inferior to those of Table 21, of which seeds of commom fruits were employed (see Tables XXI and XXII in the appendix).

The result of Table 23 is too low to derive a safe conclusion.

At any rate, the favorable effect of disaccharide in comparison with hexose is clear.

As for the concentration of the sugar, the results in Tables 21 and 22 represent an example with disaccharide and with hexose respectively. For the former, the optimum seems to exist at 5% or thereabout, while in the latter, about 2.5% seems to be best fitted for the germination. The difference may probably be due to the difference in the molecular weight of the two compounds.

d. Oxygen requirement of the embryo.

In a foregoing section (section 5, c), it was demonstrated that the whole seeds of *Euryale* are capable of germination in an oxygen free condition, and that their germination percent cannot be improved by applying full oxygen pressure. In experimenting with the germination of the isolated embryo, a similar fact was again realized.

At first, the germination of the isolated embryo under anaerobic condition was studied. The procedure was as follows: — the medium was sufficiently boiled to drive the dissolved air away, rapidly cooled, put in with the isolated embryo, and then paraffin oil was added, which forms a covering layer over the surface of the medium. Incubated as usual. For the culture medium, 0.1% KNOP's solution with 0.05 g. asparagin and 2.5 g. saccharose was employed. Control test means the culture in the medium neither boiled nor paraffined. The results are shown in Table 25.

TABLE 25.   Germination under anaerobic condition.

Material, seed of the autumn of 1927 Date of exp., 19/I, 1928.

Oxygen relation	Anaerobic	Control
Germ. pct. after 45 days	100%	100%

The capability to germinate in anaerobic condition was decidedly demonstrated, corroborating the behavior of the whole seeds mentioned in the previous section.

In the above experiment, the control test was conducted with

embryos which were simply put in the culture media in the test tube.
Thus treated, the embryos sank ordinarily to the bottom of the media,
which condition made the supply of oxygen rather hard. Now, it is
known that such condition may sometimes prove inhibitory to germi-
nation, and YOSHII (1925, p. 95) achieved the dismissal of this un-
favorable circumstance in his culture of immature embryos by keeping
them at the surface layer of the medium where a rather free supply
of air is allowed. His technique consists in keeping the embryo
stationary at the said position by means of a small funnel of filter
paper. My study was now extended to test if the same contrivance
may improve the germination of isolated embryo of *Euryale*. The
experiment was made with culture medium of the composition in
Table 19. The embryos were kept at the surface layer by the above
described method. As a control test, embryos in totally anaerobic
condition as in Table 25 was studied. The results are shown in the
table below.

Table 26. Oxygen relation of the embryo.

Material, seeds of the autumn of 1927 Date of exp , 19/1. 1928

Oxygen relation	Aerobic	Anaerobic
Germ. pct after 45 days	27%	33%

It is indicated that the embryo is rather indifferent to oxygen supply,
corroborating again the results in the previous section (pp. 64–66).

e. Acidity of medium in relation to germination.

In order to gain some knowledge of the influence of the reaction
of the medium on the germinative behavior of the isolated embryos,
the following experiment was carried out.

The method in general is the same as described above in p. 84.
As the culture medium, 2.5% solution of saccharose was employed,
the forcing effect of which we were already convinced of. The reac-
tion of the medium was controlled by mixing in various proportions
the two solutions, KH_2PO_4 and Na_2HPO_4 in 1/15 Mol each. (CLARK,
1923, p. 114). Four mixtures of different acidity were thus prepared.

They were sterilized in flowing steam, with a definite volume of sterile saccharose solution of known concentration added to each, just enough so as to adjust the final concentration of sugar at 2.5%. Before starting the incubation, the hydrogen ion concentration of the prepared media was determined colorimetrically, to obtain the following value : — A, Ph. .4.4, B, .Ph 5.8, C, .Ph 6.8, D, .Ph 8.2. The results of the germination test with these media are shown in Table 27.

TABLE 27. Effect of different grade of acidity.

Material, seeds of the autumn of 1928. Date of the exp., 30/I, 1929

Ph of the media	4.4	5.8	6.8	8.2	Germ pct. of whole seed
Germ pct. after 45 days	25%	50%	100%	0%	0%

In this experiment again, the forcing effect of sugar is clearly shown in contrast to the obstinate immobility of the whole seeds.

It is demonstrated by this experiment that the acidity of media exercises a marked influence on the germination. Of the four media tested, the third solution (Ph ˉ 6.8) which is approximately at the neutral point proved to be optimal, resulting in the highest degree of germination percent. On the acid side of this point, at Ph 5.8, the decreasing tendency of the forcing effect is already perceived, and at Ph 4.4, the same tendency is still accentuated. The critical point was not determined in the present case. On the other hand, towards the alkali side from the optimum, the inhibitory effect tends to increase quite rapidly, and at Ph 8.2, no embryo was capable of germination at all. The results in this section may be briefly stated : —

    1. The embryo isolated from dormant seed of *Euryale* can be forced to germinate by the application of artificial culture media.

    2. The experiments were conducted invariably at 25° in the dark.

    3. The forcing effect of the culture solution is ascribed to the sugar contained therein.

    4. Of the various kinds of sugar, saccharose, maltose, lactose, glucose and laevulose were tested, all of which except the last exhibited quite high capacity to force the embryo.

    5. The optimal concentration is about 5% for saccharose and

about 2.5% for glucose.

    6. The embryo is also capable of germination in anaerobic condition. Contrivances to facilitate the oxygen supply failed to improve the germination percent. The results coincide well with those obtained with the whole seeds.

    7. The forcing effect is demonstrated to be most intense at about the neutrality. Towards both acid and alkali sides, the effect tends to be reduced, and especially so in the latter side.

<h3 style="text-align:center">11. FORCING METHODS *</h3>

    Methods of artificially forcing the dormant seeds to germinate, or of improving the percentage of germination of seeds which are refractory to the ordinary germinative conditions have been exhaustively treated by many investigators (see the review in CROCKER, 1916, pp. 117-9).

    As is described already in the foregoing sections, the cause of delayed germination of *Euryale* seeds is to be looked for in the properties of the embryo itself and not of the seed coat. Therefore, of all the forcing methods hitherto reported, such are not worth consideration as concern exclusively the coat characters, e. g, mechanical abrasion, carbonizing with H_2SO_4, etc. (the result of total decortication has been already treated). Other methods whose mechanism is not clearly elucidated may be studied tentatively to learn if they exercise any effect. I have carried out a few experiments up to this day along this line of problem, not yet obtaining, however, a very effective means of forcing on the *Euryale* seeds. The results, therefore, shall be mentioned but briefly.

<h4 style="text-align:center">a. Desiccation.</h4>

    The favorable effect of desiccation to improve the germination is often observed and reports of the experimental studies in this respect are not rare (LUDWIG, 1886, with *Mayaca fluviatilis ;* BATALIN, 1889, with ' Roggen '; GASSNER, 1910, 2, p. 507. with *Paspalum dilatatum ;*

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    * Forcing methods may be classified, with respect to their mode of action, in two categories: those which activate the real process of germination and those which accelerate the after-ripening. In the present section, they are arranged without regard to this point, as our knowledge concerning the problem is still quite defective.

JOSEPH, 1929, pp. 199-200, with green parsnip seeds).

This treatment does not, however, seem to fit the *Nymphaeaceae*. KINZEL (1913, p. 19) reports that seeds of *Nuphar*, *Nymphaea* and *Victoria* lose vitality soon by desiccation. A limited number of seeds at best germinated sooner than the control when they were dried for a short time with the fruit wall on.

CONARD (1905, p. 106), too, notices that the seeds of *Eucastalia* group perish readily by desiccation.

As for seeds of *Euryale*, a paper by BORNEMANN (1886) shows that they cannot be kept long alive when stored in coal powder. The cause may probably be due to the reduction in the moisture content.

ARCANGELI (1888) observed also that the *Euryale* seeds stored in dry sand exhibited a less degree of germination energy than those kept under water or in moist sand.

All these data indicate that the forcing of *Euryale* seeds by desiccation is least promising, and my own experiment adds nothing new. Seeds of the lot no. 5, after five months of storage in water at the room temperature, subjected to desiccation in a thermostat of 25°. After some days they were transferred to germinative condition (tap water, 25°, in the dark). The results are shown in Table 28, indicating that no forcing was effected.

TABLE 28.   Effect of desiccation

Period of desiccation (in days)	0	1	2	4	11
Reduction of weight (expressed in percent) of fresh weight)	0	12.2	25.4	48.6	50.7
Number of seeds tested	10	10	10	10	10
Number of seeds germinated — after 1 month	0	0	0	0	0
„ 2 months	0	0	0	0	0
„ 9 „	2	1	1	0	0
„ 1 year	0	0	0	0	0

Seeds of the same lot as in Table 28, kept dry for 100 days at 30°, or in a desiccator filled with $CaCl_2$, were tested for their germi-

nation capacity. 40 seeds each of both the treatments were studied and none of them responded to the germinative condition at all during one year of experiment. On the expiration of the period, they were dissected to find their embryos all degenerated. Thus the desiccation over a long period seems fatal to the *Euryale* seeds.

b. Salt solution.

As a germinative medium for seeds, water with some chemicals added, especially those containing nitrogen, often exercises better influences than when without such. The effect is especially remarkable with light sensitive seeds and many elaborate works have been accomplished on this phenomenon (LEHMANN, 1909, 1919; GASSNER, 1915, 1917).

As for the *Euryale* seeds, I have conducted a provisionary study in 1926, in order to learn whether any similar effect can be established with them. The results are shown below in Table 29.

TABLE 29. Effect of salt solution.

Material, lot no. 5   Duration of exp., 13/II to 15/XII, 1926. Temperature, 25°. Light, excluded.

Medium	Number of seeds tested	Number of seeds sprouted in		
		Feb.	March	Apr. — Dec.
Pure water	20	0	3	0
Pond water	10	0	1	0
KNOP's solution 0.1%	,,	0	0	0
KNO_3 solution 0.1%	,,	0	0	0
K_2HPO_4 solution 0.1%	,,	0	0	0
KCl solution 0.1%	,,	0	1	0
$Ca(NO_3)_2$ solution 0.1%	,,	0	0	0
$MgSO_4$ solution 0.1%	,,	0	1	0
KH_2PO_4 solution 0.1%	,,	0	2	0

Germinations were observed in some kinds of media, but any better result than in the pure water was hardly secured, so that it may be assumed that the addition of mineral salts seems not to be

very promising to force the seeds. Conclusive results are, however, not to be derived from this experiment owing to the shortage in the total number of seeds tested. A fact is certain, at any rate, that the *Euryale* seeds do germinate in pure water. (Besides the example in the stated table, another case of positive result in pure water was observed: 5 *Euryale* seeds of the lot no. 61 were sown on 29/II, 1927 in conductivity water kept in an ERLENMEYER flask of pyrex glass. Incubated at 25° in the dark. In twenty days, three seeds started germination.) (My former suggestion (1925) that the salt effect is to be respected seems not to be appropriate. The data reported in that paper must be explained otherwise.)

c. Acids and Alkalies.

In 1907, FISCHER reported of his study with the dormancy of *Alisma, Sagittaria*, etc., that he achieved forced germination by the application of acids or alkalies. He assumed that the mechanism of forcing is related to the embryonic character. CROCKER contradicted the view and suggested that H⁺ or OH⁻ ion may modify the colloidal state and accordingly the mechanical rigidity of the seed coat so that the imbibing embryo can elongate without much difficulty.

If the latter view is to be applied, the seeds of *Euryale*, where delayed germination has almost no concern with the coat property, are not fit to be forced by such means. The experiment described below supports this view.

Material, lot no. 5. Solutions tested, 0.3 Mol solution each; HCl, NHO₃, Lactic acid, Acetic acid, Oxalic acid and NaOH.

Treatment, the seeds were put into the solution above, kept at 30° for 15 or 22 hours. They were then transferred into tap water in ERLENMEYER flasks and incubated at 25° in the dark. 10 seeds for each solution.

Duration, 12/II to 12/III, 1926.

Result, no germination.

d. Sugar solution.

Forcing effect of sugar solution is proved by GODLEWSKI (1904) with *Lupinus* (cited by MORINAGA, 1926, p. 133), KNUDSON (1922)

with Orchid seed and IVES (1923) with *Ilex opaca*.

As for *Euryale* seeds, the favorable or almost indispensable influence of sugar on the forcing of isolated embryo is decidedly shown in the previous section. If a proper means is applied to secure the penetration of sugar into the seeds, a similar effect must be obtained with the embryos encased in the seed coat.

With this view in mind, the following experiment was undertaken. First, the seeds were sterilized in 1 : 500 corrosive sublimate solution for about five minutes, and then washed with sterile water. To secure the penetration of the sugar, a portion of the seed coat at the peri-micropylar region was scraped away with a sterilized knife. The seeds were each then put into test tubes containing 2.5% maltose solution (previously steam sterilized). Incubated at 25° in the dark. Contrary to the case with isolated embryos, the procedure was not effective to suppress the contamination of microorganisms. For, in the present case, those germs infected previously in the deeper portion of the seed coat and protected from the destructive action of the desinfectant commenced vigorous growth as soon as they were put into the sugar solution. Owing to the heavy contamination thus developed, the main aim of the study was seriously interfered with and only five positive results were obtained out of 45 seeds tested (15 seeds each from three different lots of 1928). If, however, an effective means of sterilization be devised, the result will be much improved.

e. Amylase solution.

Instead of depending on the sugar solution supply from outside, as in the preceding experiment, transformation of the seeds' own foodstuff into sugar was studied next. For this purpose, Takadiastase solution 1 : 500 was applied which was previously sterilized by filtration. The general arrangement is identical to that of the foregoing experiment. Penetration of the enzyme was secured also in the same way.

In the early stage in the incubation at 25° in the dark, contamination was already very remarkable. Notwithstanding such unfavorable circumstances, 4 seeds out of 45 germinated in from two to three weeks. The control test showed no germination in the meantime.

The result indicates that the *Euryale* seeds in amylase solution can germinate. The forcing is without doubt effected through the sugar produced by the hydrolysis of the stored starch. It may be criticized that the percentage of germination is rather poor in this experiment. Yet, it must be reckoned also that contamination could not be excluded, which much interfered with the result, and the expectation is not unfounded that the percentage would be improved but for such troublesome interference.

f. High temperature of the germinator.

For the *Euryale* seeds not·yet sufficiently aged, the temperature minimum of germination is rather high, and they never respond positively at a room temperature. If higher temperatures are applied, some seeds are observed to germinate readily, as is shown above in pp. 61–62. Such examples are, however, very rare or rather of an exceptional occurrence. It seems that high temperature is not effective enough for the present purpose.

g. Alternation of temperature.

The favorable effect of high and low temperatures applied alternately is established with various plant seeds (KINZEL, 1900, with *Larix, Holcus* and *Anthoxanthum ;* GASSNER, 1910, p. 510, with *Paspalum dilatatum*, 1911, with *Chloris ciliata ;* LEHMANN, 1911, pp. 585–589, with *Epilobium spp., Veronica longifolia ;* MORINAGA, 1926, pp. 142–145, with *Cynodon, Poa, Typha, Apium* and *Berberis ;* DAVIS, 1927, pp. 252, 255, with *Sambucus* and *Berberis ;* Joseph, 1929, pp. 202–203, with parsnip).

My study with *Euryale* seeds failed to obtain positive result with such treatment.

Experiment. Material, 2 seeds each of nos. 62, 64, 66, 69, and 610. 10 seeds in total numbers. Duration of exp. 2/II, 1927 to 1/II, 1928.

Treatment, seeds were put alternately for every 24 hours, in a thermostat of 25° and MANNESMANN's refrigerator (7°–12° in summer and 2°–4° in winter). Medium, tap water. Light, excluded.

Result, no germination.

In this experiment, too low temperature was applied in comparison with those of the former authors, so that combination of other degrees of temperature than in this experiment may result in a different way. Further experiment has not, however, been carried out.

h. Cold storage.

For those seeds whose delayed germination is caused by embryonic characters, application of low temperature in storage or in stratification period was repeatedly tried with success to shorten the dormant period (BATALIN, 1889, with ' Hafer ', DAVIS and ROSE, 1912, with *Crataegus mollis* ; ECKERSON, 1913, with *Crataegus spp.* ; ROSE, 1919, pp. 288-9, with *Tilia americana* ; JONES, 1920, pp. 139-141, with *Acer saccharum* ; PACK, 1921, 1, with *Juniperus virginiana* ; ABBOT, 1923, p. 176, with apple , WEISS, 1926, p. 741, with *Betula populifolia* ; DAVIS, 1927, with *Cornus florida* and *Sambucus canadensis* ; CROCKER, 1927, with *Rosa rubiginosa*, Damson plum, peach and French pear ; JOSEPH, 1929, pp. 132-6, with *Betula spp.*).

The similar effect with the *Euryale* seeds is suggested by the following observations. The seeds employed in the previous experiment on the forcing effect of alternation of temperature, after being tested in vain with that treatment, were left untouched thereafter in the refrigerator so that they were exposed constantly to low temperature. This condition continued from Feb. 1, 1928 to the beginning of August, when the cooling operation was compelled to be suspended owing to trouble in the supply of electricity. Soon after the elevation of the temperature thus occasioned, the seeds began to germinate and five seeds out of ten completed the germination.

Another example is the following. *Euryale* seeds, 100 in total (10 seeds each of nos. 711-720) were incubated at 30° in tap water in the dark on Dec. 28, 1927. In twelve days, a single seed germinated, and in forty-two days another. No other seeds exhibited any sign of germination thereafter. From Feb. 21 on, 'they were transfered into the refrigerator and stored there continuously in coldness. At the beginning of August, cooling was suspended as is mentioned above. The seeds began to sprout immediately, and germination as high as 63% was attained.

Further examples of similar observations are at hand, but I will omit them here for the sake of brevity.

Thus the favorable effect of cold to accelerate the after-ripening is suggested also with respect to the *Euryale* seeds. However, the state of the matter is not as yet established with sufficient clearness, for a delicate difference in other conditions seems to exercise a serious interference. A slight change in the previous treatment before cooling may greatly modify the result. For instance, a parallel experiment with the second examples of the above given two, was carried on with the seeds of the same lots of the material, to eliminate the error due to the fruit individuality The seeds were kept at 25° from Oct. 29, 1927 to Feb. 21, 1928 and then transferred to the refrigerator, where they were left until the beginning of August. After the suspension of cooling, not a single seed germinated, quite contrary to the foregoing example.

Thus we know that the difference in the earlier conditions, 30° for 55 days in the one case and 25° for 115 days in the other resulted in totally contrary effects with all the uniformity in the selection of the material and application of the coldness.

I am still ignorant at present as to which factors in these examples accelerate the germination on the one hand and retard it on the other. In 1928, it was attempted to study whether the cold storage itself is potent enough to improve the germination of *Euryale* seeds. The seeds of the autumn of 1928 were put into the refrigerator directly after the harvest, i. e., without any previous treatment. The germination test (25°, in tap water, in the dark) with these seeds at intervals up to this date revealed that the storage in MANNESMANN's refrigerator (monthly average of daily maxima and minima is : — in Feb., 2°-4°, in June, 6°-9°) for six months is not effective enough to improve the germination except those of a few special fruits. Control test with the seeds of the same material stored at the temperature of the laboratory room or at a very low temperature (from −11° to −16°) for the same period resulted also negatively. The study is now going on further, and I hope the result will be reported at a later date.

In short, the favorable effect of cold storage for the after-ripening is highly probable and this is one of the most promising methods of accelerating the after-ripening process. Determination of the optimum

temperature, and the time requisite for completion of the after-ripening
is left for future study.

Application of very low temperature 50 seeds of the lot no. 80
were put into liquid air, left undisturbed for 16 hours (from 17 hour
Dec. 18, 1928 to 9 hour in the next morning), and then transferred
into tap water at 25° in the dark, to study if they were accelerated.
No positive response was obtained in the course of one month in the
germinator (germination percent of the control test, 6/50 = 12%).

1. Control of oxygen supply.

We have treated the matter already in previous section and
demonstrated that the *Euryale* seeds are rather indifferent with respect
to oxygen. It was shown there that the forcing result was effected
neither by the increase nor the reduction in oxygen supply.

12 CONCLUSION

Scientific works devoted to the study of the delayed germination
of plant seeds amount to a considerable number up to this date, and
the inspection into the mechanism was achieved by many authors to
greater or less degrees, as is briefly reviewed at the outset of the
present paper. According to the classification of CROCKER (1916),
the cause of delayed germination is ascribed in the main to five
different conditions in the seed, viz., two embryonic and three seed
coat characters.

Now, in the case of *Euryale* seeds, of which the delayed germina-
tion is established without doubt (section 4), the cause responsible
seems to be looked for in the embryonic characters. For, when the
cause lies in the coat characters, it must be conditioned by the water
exclusion, or the oxygen impermeability, or mechanical resistance of
the seed coat, none of which seem to fit the case of *Euryale* seeds.
Furthermore, the decortication is totally ineffective to secure germina-
tion. Then the cause is to be looked for in the embryonic character,
either in the morphological or in the chemical. From the morpholo-
gical point of view, however, the embryos of *Euryale* exhibit no
fundamental difference between when freshly harvested and after a
period of aging. They are well developed and differentiated from

the start, when they leave the mother plant   Accordingly, the delayed germination cannot be explained from this point.

The last possibility left is the delayed germination due to the chemical nature of the embryo. Studies along this line revealed that some changes in the composition of the embryo take place during the course of the after-ripening. The appearance of reducing sugar is one of the most remarkable distinction. Other parallel transformations are highly probable, but not fully studied yet. At any rate, it is a fact without doubt that the germination percentage is improved with the appearance of the sugar   It is suggested thus that there must be some parallel relation between the two phenomena, and it is not very inconsistent if the existence of sugar be presumed as a condition for causing a prompt germination.

The verity of this view is demonstrated furthermore by the study with embryos isolated from the seeds. It was proved that the embryos isolated from the still dormant seeds can be forced to germinate only when supplied with sugar solution.

Taking these circumstances into consideration, it seems that in the after-ripening process of Euryale seeds in the natural condition, the production of sugar takes place at some stage which then acts to awake the embryo. As to whether this relation is a direct one or needs some connecting links between, I am still ignorant. Nor is determined the condition which necessarily precedes the production of the sugar.

Similar observation of the relation of the sugar and germination capability in the dormant seeds have hitherto been reported in many species, and the present example represents certainly another analogous case. The fact that the forcing of the embryo was experimentally achieved by the application of sugar may be regarded as a contribution of the present paper to the knowledge on the delayed germination.

It must be mentioned here also that in some exceptional cases Euryale seeds can germinate without aging or the production of reducing sugar. In such cases, however, the requirement for the germinative condition is quite high as compared with the case of fully aged ones.

Lastly a brief mention will be given concerning the period of the after-ripening. Contrary to those seeds of which the delay of germi-

nation is caused by the coat character, those seeds with truly dormant embryos cannot dispense with a more or less prolonged period of after-ripening through which the necessary preparatory changes take place. In order to shorten this period, proper conditions must be applied which fit best to accelerate the necessary change. Exposure to cold is hitherto recommended by many authors to be the most effective treatment. The few experiments with *Euryale* seeds up to this day, however, proved to be not very successful. It is only realized that the conditions are rather complicated, to control which is left for future study. If some effective means were invented which were sure to shorten the after-ripening period to a great degree, the study of the necessary embryonic change would be much accelerated, which I hope will be accomplished in the future.

13 SUMMARY

1. The present study was carried on exclusively with the seeds of *Euryale ferox* SALISB. from Zyûnityôgata.

2. The seed consists of four parts, viz., seed coat, perisperm, endosperm and embryo.

3. Four different layers are distinguished in the seed coat, viz., a) epidermal, b) sclerenchymatous, c) non-lignified and d) cork layer. The last one, although the least remarkable so far as the thickness is concerned, is nontheless quite significant for its being suberized.

4. The *Euryale* seeds freshly harvested do not germinate at the ordinary room temperature of the laboratory, nor has the application of high temperature proved of any effect.

5. Seeds after-ripened in their natural habitat have a lower grade of minimal temperature (lower than 8°). At 25°, their germination percent attains quite a high value, and at 20°, a slight reduction is observed. Higher temperature was not studied with seeds of this stage.

6. When the seeds are stored in mud and water in a hot house, or under such condition as approximates that of outdoors, the after-ripening process takes place in the seeds which then become ready to germinate. Thus some seeds sprout in the first spring after the harvest. The remaining majority, however, keep immobile at that period and germinate in the second spring as a general rule. Accord-

ingly, a dormant period of 18–20 months seems usual for *Euryale* seeds.

7. It is noticed that smaller sized seeds tend to germinate earlier. Such seeds seem to be capable of finishing after-ripening in a shorter period.

8. Seeds somewhat aged but not fully after-ripened can be forced to germinate by high temperature. Yet, the minimal temperature is still quite high (higher than 15°). Optimal point seems to be at 25° or thereabout.

9. *Euryale* seeds are not very exacting with respect to the light, so that germination incapability due to light relation cannot be accepted.

10. They can germinate under either an anaerobic or an oxygen saturated condition. They seem to be rather indifferent with respect to oxygen-relation. At least, their delayed germination is not caused by the deficiency in oxygen supply.

11. *Euryale* seeds are possessed of as much as some 50% of water, by far a larger amount as compared with that of an ordinary land plant.

12. They are fully saturated with water, and decortication effects no further imbibition, nor does preparatory water intake seem necessary for germination.

13. It is established that water can pass through the seed coat. The distribution of resistance is not uniform for different layers of the coat, the innermost one being the least permeable to water.

14. The dormancy cannot be conditioned by the water exclusion by the seed coat.

15. The force of growth of the germinating embryo and the resistance of the seed coat against it were measured. It is demonstrated that the former is by far the larger than the latter. It is shown also that the latter is variable according to moisture condition, being more resistant when desiccated.

16. Decortication test is totally ineffective to force the seed to germinate.

17. From the data so far given, it may be concluded that the coat characters are not responsible for the delayed germination.

18. The embryo of *Euryale* seed is fully established both in differentiation and in magnitude. Enlargement during the course of

after-ripening is not excluded, but such examples are rather exceptional.

    19.   Presence of starch, fat, protein and catalase is demonstrated in the seeds of *Euryale*.

    20.   Some transformation in the chemical properties are sure to take place in the after-ripening period. The appearance of reducing sugar is one of the most noticeable of such.

    21.   The embryo isolated from dormant seeds of *Euryale* can be forced to germinate by cultivation in sugar-containing synthetic medium. Among the ingredients of such medium, sugar seems to be the agent which is responsible for such an effect.

    22.   Disaccharides are more efficient than hexoses, the optimum concentration is 5% for saccharose and 2.5% for glucose.

    23.   The embryo is also capable of germination under anaerobic condition. Contrivance to facilitate the oxygen-supply failed to improve the germination percent.

    24.   The forcing effect seems to be most intense at about neutrality. Towards both acid and alkali sides, the effect tends to be diminished, and especially so in the latter side.

    25.   As methods of forcing the seeds, the following treatments were tested, viz., desiccation, salt solution, acid and alkali, sugar solution, amylase solution, high temperature, alternation of temperature, cold storage and control of oxygen-supply. With all of these treatments, any decidedly effective result has not yet been attained up to the present time. The most promising is the application of cold storage. Even in this case, I am yet unable to master the mode of action and am still ignorant of the optimum temperature, the time requisite for completion of the after-ripening, etc. Application of sugar or amylase solution may prove effective if proper means to suppress contamination be invented. As for the effect of high temperature, see Article 8 in the above lines. All the other treatments do not seem to be very suitable as forcing methods.

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15. APPENDIX.

The numbering of the tables in this section is so arranged that the numerals here (in Roman letters) each correspond to the same in the foregoing sections (in Arabian letters). For instance, Table XX here corresponds to Table 20 in p. 86; the latter representing the concise form derived from the data described in detail in the former. The discontinuity of the numbering here is due to the above circumstance.

TABLE I, a.

			Whole seed			
Lot	Height	Width	Width/Height	Fresh Weight	Dry Weight	Dry Wt./Fr.Wt.
61 a	13.5	12 4	91 85	1.316	0.6822	51.81
61 b	14.0	12 9	92 15	1.438	0.7428	51.64
61 c	14.0	12.9	92.15	1.470	0.7330	49.87
61 d	13.7	12 8	93.44	1.371	0.7041	51.35
61 e	13.9	12.8	92.08	1.440	0.7320	50.83
63	11.5	11.0	95.65	0.924	0.5311	57.50
64	13.3	12 3	92.47	1.204	0.4991	41.50
66	12.7	11.6	91.35	1.098	0.5198	47.3
67	12.5	12.2	97 61	1.227	0.6981	56.9
69	13.4	12.1	90.30	1.360	0.6956	51.5
Average	13.25	12 30	92.91	1.284	0.6538	51.0

TABLE I, b.

Lot	Seed coat			Perisperm		
	Fresh Weight mg.	Dry Weight mg.	Dry Wt./Fr.Wt. %	Fresh Weight mg.	Dry Weight mg.	Dry Wt./Fr.Wt. %
61 a	688	309	44.89	619	370	59.96
61 b	749	341	45.54	680	399	58.71
61 c	769	323	42.04	691	407	58.91
61 d	727	333	45.82	625	369	58.15
61 e	760	333	43.83	670	396	59.08
63	495	253	51.09	422	276	65.40
64	642	203	31.65	554	294	53.03
66	548	203	37.96	541	309	57.20
67	636	319	50.08	582	377	64.77
69	719	312	43.35	622	381	61.32
Average	673.4	293.4	44.126	601.5	357.9	59.645

TABLE I, c.

Lot	Embryo				Proportion of the parts (in fresh wt)			
	Length mm.	Fresh Weight mg.	Dry Weight mg	Dry Wt./Fr Wt %	Whole seed	Seed coat	Peri-sperm	Embryo
61 a	2.9	9.1	2.8	31	100	52.3	47.0	0.7
61 b	2.9	9.3	2.7	29	100	52.1	47.3	0.6
61 c	2.9	10.7	2.9	27	100	52.2	47.0	0.8
61 d	2.9	9.2	2.8	30	100	53.0	46.3	0.7
61 e	2.9	9.3	2.7	29	100	52.8	46.6	0.7
63	2.6	7.0	2.2	31	100	53.6	45.7	0.8
64	2.6	7.7	2.0	26	100	53.3	46.0	0.6
66	2.5	9 0	2.4	27	100	49.9	49.3	0.8
67	2.7	9.0	2.6	29	100	51.8	47.4	0.7
69	2 6	9.1	2.6	29	100	53.3	46.1	0.7
Average	2 75	8.94	2.57	28.8	100	52.43	46.86	0.71

TABLE VI.

	15°		20°		25°		30°	
	Number of seeds tested	Number sprouted	Number of seeds tested	Number sprouted	Number of seeds tested	Number sprouted	Number of seeds tested	Number sprouted
no. 61	5	0	5	3	12	11	5	2
no 62	5	0	5	1	7	1	5	0
no 63	2	0	- -	—	6	2	1	0
no 64	4	0	—	—	5	0	1	0
no 66	5	0	5	3	7	6	5	0
no 67	5	0	—	—	4	1	1	0
no. 69	4	0	5	1	7	0	5	0
no 610	3	0	5	0	7	0	4	0
Total	33	0	25	8	55	21	27	2
Germ. pct.	0%		32.0%		38.2%		7.4%	

TABLE XX.

Lot	Number of seeds tested	Medium	Counts after 45 days							Germination of whole seed
			I	II	III	IV	V	VI	VII	
no. 711	8	###	3⎫	5⎫	2⎫	2⎫	0⎫	0⎫	0⎫	
		++	2⎬8	2⎬8	3⎬8	3⎬3	0⎬0	0⎬0	0⎬0	posit. 0
		+	3⎭	1⎭	3⎭	0⎭	0⎭	0⎭	0⎭	
		−	0	0	0	1	7	8	8	negat. 10
		cont	0	0	0	4	1	0	0	
no. 713	6	###	3⎫	3⎫	1⎫	1⎫	0⎫	0⎫	0⎫	
		++	0⎬4	2⎬5	1⎬6	3⎬5	0⎬0	0⎬0	0⎬0	posit. 0
		+	1⎭	0⎭	4⎭	1⎭	0⎭	0⎭	0⎭	
		−	1	1	0	1	6	6	6	negat 10
		cont	1	0	0	0	0	0	0	
no. 715	6	###	1⎫	4⎫	1⎫	0⎫	0⎫	0⎫	0⎫	
		++	1⎬4	2⎬6	1⎬2	0⎬0	0⎬0	0⎬0	0⎬0	posit. 0
		+	2⎭	0⎭	0⎭	0⎭	0⎭	0⎭	0⎭	
		−	1	0	3	6	5	6	6	negat 10
		cont	1	0	1	6	1	0	0	
Total	20	posit	16	19	16	8	0	0	0	posit 0
		negat	2	1	3	2	18	20	20	negat 30
		germ pct	88.9	95	84.2	80	0	0	0	germ pct. 0
Composition of media		Knop's sol	+	+	−	−	+	+	−	
		Asparagin	+	−	−	+	+	−	−	
		Saccharose	+	−	+	+	−	−	−	

TABLE XXI.

Lot	Number of seeds tested	Medium	Counts after 45 days				Germination of whole seed
			I	II	III	IV	
no. 711	3	−	3	### 3	### 1⎫ + 1 cont. 1	+ 3	posit 0 negat. 10
no. 712	3	−	3	− 2 cont. 1	### 2 + 1	### ʻ1⎫ + 1	posit. 0 negat. 10
no. 713	3	−	3	### 1 + 2	+ 2 − 1	− 3	posit. 0 negat. 10

TABLE XXI. contd.

Lot	Number of seeds tested	Medium	I		II		III		IV		Germination of whole seed	
no 714	3	—	3		### 2		++ 1		++ 1		posit	0
					cont. 1		+ 2		— 2		negat	10
no 719	3	—	3		+ 1		— 3		— 3		posit	0
					— 2						negat	10
Total	15	posit 0			. 9		... 10		6		posit.	0
		negat 15			. 4		... 4		. 9		negat	50
		germ pct 0			69.2		71.6		. 40		germ pct	0

TABLE XXII.

Lot	Number of seeds tested	Medium I	II	III	IV	V	VI	Germination of whole seed	
no 711	3			+ 3	+ 3	+ 3	+ 2 / — 1	posit	0
								negat.	10
no 713	3			+ 3	+ 1 / — 1 / cont 1	+ 2 / — 1	— 3	posit.	0
								negat.	10
no 714	3	no germination	no germination	+ 1 / — 2	++ 1 / — 2	+ 1 / — 2	— 3	posit.	0
								negat.	10
no 715	3			+ 1 / — 1 / cont. 1	+ 1 / cont 2	+ 3	+ 1 / — 2	posit.	0
								negat	10
no 716	3			+ 2 / — 1	++ 1 / — 1 / cont 1	+ 2 / — 1	— 3	posit.	0
								negat.	10
no 720	3			— 3	— 1 / — 1 / cont 2	+ 1 / — 2	— 3	posit.	0
								negat.	10
Total	18	posit. 0	.. 0	10	 7	... 12	... 3	posit.	0
		negat. 18	..18	.' .7	 5	 6	15	negat.	60
		germ. pct 0	.. 0	....58.5	....58.3	... 66.6	....16.6	germ. pct.	0

TABLE XXIII.

Lot	Number of seeds tested	Counts after 45 days		Germination of whole seed	
		I Laevulose	II Maltose		
no. 714	3	− 1	+ 1	posit.	0
		cont 2	cont 2	negat	10
no. 725	4	+++ 1	− 3	posit.	0
		cont 3	cont 1	negat.	10
no. 736	4	− 4	++ 1	posit	0
			− ½		
			cont 1	negat.	10
no. 738	4	− 4	+++ 2	posit.	0
			− 2	negat.	10
no. 740	5	− 5	+++ 1	posit.	0
			− 4	negat.	10
Total	20	posit. 1	 5	posit	0
		negat 17	 11	negat.	50
		germ pct 5.1	.. 31.3	germ. pct.	0

TABLE XXIV.

Lot	Number of seeds tested	Counts after 45 days			Germination of whole seed	
		Saccharose	Maltose	Lactose		
no 811	8	+++ 1	+++ 1	++ 4	posit.	0
		++ 1	++ 6	+ 2		
		+ 5	− 1	cont. 2	negat.	8
		− 1				
no. 817	10	++ 3	+++ 2	++ 3	posit.	0
		+ 4	++ 6	+ 4		
		− 2	+ 2	− 2		
		cont. 1		cont. 1	negat.	10
no. 819	10	++ 3	+++ 2	++ 5	posit.	1
		+ 6	++ 5	−+ 4		
		+ 1				
		cont 1	cont. 2	− 1	negat.	9
Total	28	posit 23	... 25	 22	posit.	1
		negat. 3	 1	 3	negat.	27
		germ. pct. 89.2	.... 96.2	 88	germ. pct.	3.6

TABLE XXV.

Lot	Counts after 45 days				Germination of whole seed	
	Anaerobic		Control			
	⧻	3	⧻	3		
	+	2	⧗	2	posit.	0
	−	0	+	3		
no. 711			−	0	negat.	10
	total	5	total	8		
	germ pct	100	germ pct.	100	germ. pct.	0

TABLE XXVI.

Lot	Number of seeds tested	Counts after 45 days		Germination of whole seed	
		Aerobic	Control (anaerobic)		
no. 725	3	posit. ... 1	 0	 0	
		negat. 2	... 3	 10	
no 735	3	posit ... 0	... 3	... 0	
		negat ... 3	.. 0	 10	
no. 736	3	posit. . . 1	. . 0	... 0	
		negat. ... 2	... 3	 10	
no. 738	3	posit . 2	... 1	 0	
		negat. 1	.. 2	 10	
no 740	3	posit. ... 0	. 1	 0	
		negat ... 3	.. 2	 10	
Total	15	posit. ... 4	 5	 0	
		negat. 11	 10	 50	
		germ. pct. 27	 33	 0	

TABLE XXVII.

Lot	Number of seeds tested	Counts after 45 days				Germination of whole seed	
		A $P_H =4.4$	B $=5.8$	C $=6.8$	D $=8.2$		
no. 820	10	⧗ 0⎰3 ⧗ 3⎱	2⎰3 1⎱	2⎰10 8⎱	0	posit.	0
		+ 3⎱3	1⎱3	8⎱10	0		
		− 7	7	0	10	negat.	10

TABLE XXVII contd.

Lot	Number of seeds tested	Counts after 45 days				Germination of whole seed	
		PH	A =4 4	B =5 8	C =6 8	D =8 2	
no 821	10	++	1½}2	7½}7	6½}10	0	posit	0
		+	1½	0½	4½	0	negat	10
		—	8	9	0	10		
Total	20	posit	5	10	20	0	posit	0
		negat.	15	10	0	20	negat	20
		germ pct	25	50	100	0	germ pct.	0

EXPLANATION OF PLATE

Fig 1   Embryo cultivated in artificial medium   Material, embryos of lot no 62
       Composition of medium 0 1% KNOP s solution 100 cc Asparagin 0 05 g
       Saccharose 2 5 g   Temperature, 25° Photographed on 45th day from
       the start of the experiment (see Table 19)
Fig 2.   Do   The samples are those recorded in Table 20   The numerals in
       Roman characters correspond to those in the same table
       II   KNOP s solution with saccharose
       III   Simple saccharose solution
       IV   Saccharose solution with asparagin but without KNOP's solution
       VI   Simple KNOI s solution
       VII   Tap water
       Photographed on the 45th day from the start of the experiment
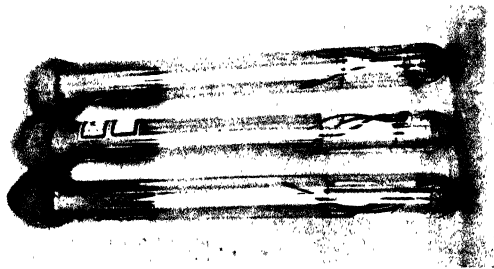
Fig. 2.

Fig. 1.

On a Fungus Found in the Urine and the Cerebro-spinal Fluid of a Patient Suffering from Meningitis

By

Toshio Ohue

(B olog al Laboratory Matsuyama High School Eh me Japan).

(W th Pl VI VIII and 3 Text figures)

Dr W Sakai found a few spores in the urine and cerebrospinal fluid of a patient who was suffering from meningitis The following statements were given by Dr Sakai concerning the symptoms and the course of illness

Mr W aged 59 No remarkable anamnesia One night he suddenly felt severe headache and dizziness in a half hour after eating of a few boiled eggs and dried persimmons Soon after he vomited repeatedly a large amount of stomach contents and at last swooned for about 3 hours Temperature 37 8°C Three days after illness the urine was examined which was strongly acidic in reaction and 1 008 in the specific gravity The urine was clear but brownish yellow in color and contained no cellular elements except a few spores The protein reaction was negative These spores were obclavate, brownish yellow and transversally and longitudinally septated Their size was 7 35 micra in length and 7 12 micra in breadth The headache did not disappear in this day and the rigidity of the neck and Kernic s sign were also observed Ten days after the pathogenesis a spinal puncture was made The liquid dropped somewhat quicker than in the normal person and was clear in appearance and yellow in color Microscopically were seen many one or two celled yellowish organisms These organisms were cultured and produced spores 27 days after the pathogenesis the rigidity of the neck disappeared tactile sense became normal, Kernig's sign disappeared, Romberg's sign became negative. The patient slept morbidly since the falling to ill The spores were detected in the urine in the 3rd, 5th, 6th, 8th, 9th, 12th 15th 27th 28th and 32nd to 36th days. The spores were found in the spinal

fluid even after 38 days. From the dried persimmons which the patient had eaten many spores similar to those contained in the urine were found. The patient left the hospital before complete recovery.

It seems as though the spores were some way connected with the cause of the present disease. Even if it be not so, it is interesting to follow the exact course of the circulation and excretion of such large spores in the animal body. For the reason just mentioned I have carried out the investigation concerning the nature of those spores mycologically and pathologically.

Here I wish to express my hearty thanks to Prof. S. HATAI under whose permission the present work was undertaken, to Prof. M. OTA for his kind guidance and encouragement which helped me throughout the entire course of the work, and to Dr. W. SAKAI for the kind bestowment of the valuable materials. I am also much indebted to Prof. S. NASU for his valuable advice and kind criticism.

MYCOLOGY

1. CULTURAL CHARACTERS.

The pure culture of the spores used for the present investigation was obtained from the urine of the patient. The urine was centrifugarized and the spores thus collected were washed repeatedly with 2% solution of hydrogen peroxide for a few minutes, and were transfered to the cultural media. Since it was a question whether those spores belong to one species or are a mixture of different species, it was necessary to obtain the culture from each single spore. For this purpose I followed BOLLE's method of single spore isolation.

Although the fungus grows on all ordinary culture media, especially when sugars are present, best results were obtained on persimmon agar or banana agar, in which it produces a vigorous growth of air mycelia. The culture media becomes pigmented in company with their growth. Zonation frequently appears, but is more clearly marked with the media containing relatively less sugar. The cultural characters have been studied upon a number of different media. I shall describe these in detail, based on the observations made with SABOURAUD's glucose agar.

On SABOURAUD's glucose agar plate, conidia begin germinating on an average of from to six germ tubes, which are hyaline at first, but later become dark. Thalli become macroscopic in one or two days and attain a diameter of about 7 cm. in 8 days at a temperature of 22°C. The color of the thallus is lighter in the media which are rich in sugar. In such media, the formation of whitish aerial mycelia becomes vigorous and conidia formation is more or less arrested. The color of the mycelium becomes darker with age. The sporulation always occurs within three days after culture, under ordinary laboratory conditions. Usually the central portion of the colony is dark grayish olive in color, and around it is a zone of lighter colored mycelia about 1 cm. wide. (Plate II, 7).

In the urine, conidia germinate within 6 hours and mycelial development is observed, although conidia formation does not usually occur.

The degree of resistance to high temperature is remarkable. At a temperature of 38°C., the development becomes very slow and weak, but the conidia formation is not yet arrested. At 46°C. germination can still take place though the growth ceases after a few hours, and no further growth is shown even after it is returned to the room temperature. The degree of tolerance against acid or alkali by this organism was also remarkable. The results of several trials indicate that within the ranges between pH 4 and pH 8.5 there is no appreciable difference in the rate of growth.

2. MORPHOLOGY

a) Mycelium. The mycelium varies greatly on different media. On persimmon agar the diameters of the hyphae range from about 3 micra to 8 micra but those in the urine range from about 2 to 5 micra. On the persimmon agar the cells are usually filled with a highly refractive contents resembling oil droplets. The color of the mycelium is hyaline when young, but later it becomes a yellowish brown.

b) Conidiophores. Condiophores on the persimmon agar are dark brown, and not very greatly differentiated from the mycelium, and as a rule unbranched. They are 3 to 5 celled. (Plate I, 1–5).

c) Sclerotioid body. Sclerotioid bodies were not formed in any artificial cultures.

d) Conidia. Conidia are borne at the tip of the conidiophores. Those produced on media are usually connected; on an average, 2 to 8 forming the chain (Plate I, Fig. 2). They are obclavate brown, and 4–76 × 4–16 micra in age with from 1 to 10 transverse septa and 0–6 longitudinal septa. Conidia terminate in a hyaline, septate beak. The length of the beak varies from one ﬁfourth to one half that of the conidium. (Plate I, 1), The length of the comdia varies also considerably in different strains and on different cultural media. Tables I, II and Fig. 1 show this relation in detail.

TABLE I.   Lengths and widths of conidia formed on various cultural media.

	Media used	Mean	Mode	Standard deviation	Max	Min	Number measured
Length (in μ)	Persimmon agar	27.00±0.84	20.5	8.43	61.2	3.6	100
	Banana agar	31.58±0.82	30.5	8.16	61.2	14.0	100
	Apricot agar	21.45±0.04	21.5	0.37	23.3	10.8	100
	Bouillon agar	25.45±0.33	23.5	3.32	37.5	5.5	100
Width (in μ)	Persimmon agar	10.26±0.08	7.5	0.79	14.5	3.5	100
	Banana agar	9.91±0.04	11.5	0.42	11.5	7.5	100
	Apricot agar	8.20±0.02	7.5	0.18	10.8	3.6	100
	Bouillon agar	10.65±0.16	10.5	1.58	14.4	3.6	100

TABLE II.   Lengths and widths of conidia of 3 strains formed on the persimmon agar.

	Strains	Mean	Mode	Standard deviation	Max.	Min.	Number measured
Length (in μ)	I	29.38±0.37	25.2	8.25	61.2	10.8	500
	II	25.29±0.33	25.2	7.85	50.4	7.2	500
	III	26.78±0.33	25.2	7.43	50.4	10.8	500
Width (in μ)	I	10.67±0.07	10.5	1.66	14.5	3.6	500
	II	8.82±0.04	10.5	0.97	14.5	3.6	500
	III	10.40±0.04	11.5	0.86	11.5	3.6	500
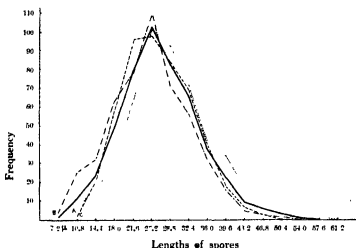
Fig. 1. Lengths of conidia of 3 different strains formed on the persimmon agar

3. TAXONOMY.

The characteristics above mentioned denote that this fungus belongs to the genus Alternaria. The specific identification of this genus must be made with the materials collected directly from the natural parasitic condition, for the fungi of this genus show various deviations from their specific characters on the artificial cultural media, as BOLLE ('24), TOGASHI ('26), ANGELL ('29) and many other authors have already indicated. In my case, therefore, specific identification was very difficult, in as much as the present investigation was carried out exclusively upon the cultured specimens. Although exact identification must await a further investigation, from the close agreement of the cultural characters I venture to state that the fungus studied by me is identical to Alternaria tenuis Nees. The literature on the Alternariosis of human and animal is very scant so far as I am aware. MATTA ('26) has reported a case of human otomycosis caused by Alternaria tenuis. WAHL and HADEN ('23 after BRUMPT) also found the spores of Alternaria sp. in the phlegms and lung tissues of tuberculosis patients in three cases.

PATHOGENECITY.

The pathogenecity of the fungus was tested with pure cultures obtained from a banana media.

1. MATERIALS AND METHODS

The animals used for these experiments were rabbits, guinea pigs and albino rats; mice and frogs occasionally were also used. The spores of the fungus were given per os 2–5 platinum ears with meal in proportion to the size of the animals. For the purpose of injection, I prepared a suspension containing about 400,000–1,000,000 spores per 1 cc. in the physiological salt solution, and 0.5–1.0 cc. was injected subcutaneously, intraperitoneally, intravenously once or intracraneally once in each animal. Ultrafiltrates of about 60 days old bouillon culture were used for the test of toxin production of the fungus. 0.5 cc. of the filtrate was introduced intraperitoneally to the albino rats and mice. For a comparison, I injected also an Alternaria species which was collected directly from decaying leaves of the poppy. The pure culture of my fungus, which was sterilized with heat, was also intravenously injected. It was my intention to investigate whether or not the spores produce a mere physical damage such as a simple foreign body like carbon powder or silicic sand, etc., produced.

2 RESULTS OF THE INJECTIONS.

Soon after an injection, the rabbits, guinea pigs and albino rats became distressed, showed less appetite, and slept deeply at a corner of the cage, thrusting their noses into the nest. In the course from two to four days the animals became lean, their fur grew ruffled, the place of injection as well as the lymph nodes of the body surface swelled, and they breathed with difficulty. The animals grew weak and cachectic by and by, and lay on one side in varying periods of from 5 hours to two days before death. The rabbits died within about 16 days, the guinea pigs within about nine days and the albino rats within about 40 days after the injections. The mortality rates were 67% in the guinea pig, 43% in the rabbit and 23% in the albino rat. (Table III).

The animals injected both with the sterilized culture and the culture of Alternaria sp. as well as those fed with the spores remained aparently

TABLE III. Result of injection experiment on the laboratory animals.

Animals		Injected materials	Seat of injection	Day on which animal died	Day on which animal was killed	Mortality rates
Rabbit	1 ♂	Culture of Alternaria tenuis (?) 1.0 cc.	intravenous	16		
,,	2 ♀	,, 1.0 cc.	,,	16		
,,	3 ♂	,, 0.5 cc	intracraneal	13		
,,	4 ♂	,, 1.0 cc.	intravenous		14	43%
,,	5 ♂	,, 1.0 cc	,,		48	
,,	6 ♀	,, 1.0 cc	subcutaneous		24	
,,	7 ♂	,, 1.0 cc	,,		40	
Rabbit	8 ♀	Culture of Alternaria sp. 1.0 cc	intravenous		14	0
,,	9 ♂	,,	,,		36	
Rabbit	10 ♂	Sterilized cult. of A tenuis (?) 1.0 cc	intravenous		18	0
,,	11 ♀	,,	,,		20	
,,	12 ♂	,,	,,		30	
Guinea pig	1 ♂	Culture of A. tenuis (?) 0 5 cc	intraperitoneal	8		
,,	2 ♀	,,	,,	8		
,,	3 ♀	,,	subcutaneous	9		67%
,,	4 ♂	,,	,,		50	
,,	5 ♀	,,	,,		30	
,,	6 ♂	,,	intracraneal	3		
Guinea pig	7 ♂	Steril cult. of A tenuis (?) 0.5 cc.	intraperitoneal		22	0
,,	8 ♀	,,	subcutaneous		26	
Guinea pig	9 ♂	Cult. of Alternaria sp. 0.5 cc.	intraperitoneous		26	0
,,	10 ♂	,,	subcutaneous		30	
Albino rat	1 ♂	Cult. of A. tenuis (?) 0.5 cc	intraperitoneal	22		
,,	2 ♂	,,	,,	26		23% *
,,	3 ♀	,,	subcutaneous	31		
,,	4 ♀	,,	,,	18		

Animals	Injected materials	Seat of injection	Day on which animal died	Day on which animal was killed	Mortality rates
Albino rat 5 ♂	Cult. of A. tenuis (?) 0.5 cc.	subcutaneous	40		
,, 6 ♂	,,	intracraneal		25	23% *
,, 7 ♂	,,	,,		40	
Albino rat 8 ♀	Filtrate of bouillon cult of A tenus (?) 0.5 cc.	intraperitoneal	2		
,, 9 ♀	,,	,,	2		
,, 10 ♂	,,	,,	1		67%
,, 11 ♂	,,	,,	3		
,, 12 ♂	,,	,,		5	
,, 13 ♀	,,	,,		5	

* The other albino rats which did not die were omited from this table

well during a period of 18 to 50 days, after which period these were killed and histopathologically examined.

The intraperitoneal injection with 0.5 cc. of the ultrafiltrate of the bouillon culture was fatal to mice and albino rats. The mice became less active soon after the injection of the filtrate. 12 to 16 hours later they sat and slept quietly in cages. By and by they grew weak, finally lying on one side with difficulty of breath before death. The animals died within 2–3 days after injection. This fact may be taken to prove an exotoxin production of the fungus.

3   CIRCULATION OF SPORES IN THE ANIMAL BODY.

It is an interesting fact that the injected spores were found usually in the urine. Dr. SAKAI detected, as already mentioned, the spores in the urine of the patient even so long as about 40 days after the onset of the illness. He has also detected a few spores in the urine of a spore fed cat.

I also succeeded in finding spores in the urine of' many injected animals, though I failed to find any in the spore fed animals.

In the frog we obtained a rather doubtful result on this point. A frog was etherized and spores were injected intraperitoneally and then the frog was fixed on a wooden dissecting board. In order to

collect the urine, a fine glass tube was inserted into the cloacal opening, and its opposite end introduced into a small test tube. The whole apparatus was covered with a large glass jar whose inside wall was covered with filter papers saturated with water. The etherization was repeated according to necessity. The fluid of the test tube was examined about 12 hours later, and only one spore was observed. There is, however, a chance to mix the urine with the rectum content in this experiment, so the present result is still doubtful without repeating the test several times.

The histological examinations carried out on rabbits, guinea pigs and albino rats showed that the spores were found most frequently in the lungs after a subcutaneous and intraperitoneal injection. The presence of spores in the encephalon was observed only in one case. The spleen, liver and gall bladder contained the spores in a few cases. I also found the spores in the kidney after the intraperitoneal injection, but their location in the tissue makes us suspect that these were accidentally removed from outside of the tissue during histological procedures, since these spores are often placed on the top of the sections but not in the tissue.

Two rabbits were killed two to three hours after the intravenous injection and were histologically investigated. The spores were found in capillaries of brains, lungs, kidneys, livers and spleens as emboli. The number of spores found in tissues was reduced greatly with lapse of time, and the detection of spores one month later was usally negative in any organs. The detection of spores by intracranial injection was also negative in all the organs except the brain.

The spores were found most abundantly in the lymph nodules situated on the omentum near the spleen, which were much swelled and firm and dark gray in color. Microscopically, numerous spores were in the hyperplastic connective tissue and were either surrounded by leucocytes or captured by giant cells.

4. PATHOLOGICAL FINDINGS.

The autopsy findings and histopathological changes of some representative cases are as follow.

Rabbit 1. male. Injected intravenously with 1.0 cc. of suspension. Died 16 days after the injection. Body weights, 1899 g. before injec-

tion and 1770 g. at the end.

The liver was considerably enlarged, with thickened borders, and was dark brown in color. The cut surface was tender and grayish yellow in color. The spleen was enlarged; edges rounded; pulp, black and spoted. The kidneys were normal in size, dark red in color, the consistency somewhat increased.

In the thoracic cavity, there was a small amount of turbid, reddish fluid. Costal pleura was moist and pinkish. Anterior right and left lobes were loosely adhered to costal pleura. The lungs were markedly nodulated and the cut surface was occupied with the densely scattered whitish nodules. (Plate III, 11). A caseinous substance mixed with dark reddish fluid was squeezed from the cut surface. The heart was normal in size. The myocardium appeared rather pale and anaemic. The blood vessels of the brain were distended and filled with blood.

Histopathology: The boundary of the liver cells was considerably obliterated. The central veins of acini were distended and filled with liquid blood. The spleen showed an increase of free cells in pulp, and endotherial proliferation in sinus. (Fig. 2). The kidneys were somewhat hyperaemic. The air space of the alveoli of lung were filled with the exudate containing a large mass of leucocytes, histiocytes and desquamated alveolar epithelial cells. The active regeneration of the latter were seen in many alveoli. The septa of the alveoli were infiltrated with white blood cells and histiocytes. Many of the histiocytes were deposited abundantly with fine and brown pigment. (Plate II, 10). In some alveoli, the exudate
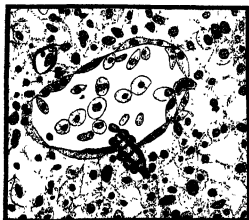
Fig. 2. Endothelial proliferation of splenic sinus. (×600)

became a homogeneous, reddish substance in which the granulation tissues were invading. The insular necrosis were seen in places.

(Plate III, 13 and 14). The nuclei disintegrated into a number of particles which were scattered, forming an island before they gradually vanished. The leucocytes accumlated at the periphery around the necrotic area. The spores were found more frequently in such a portion than in another. The spores were surrounded by histiocytes or were captured in giant cells. (Plate III, 15). The pleura situated on such severely affected parts were markedly thickened, upheaved with exudated fibrin and infiltrated with leucocytes and histiocytes. (Plate III, 12).

Rabbit 3. male. Injected intracranially with 0.5 cc. of suspension. Died 12 days after injection. Body weights, 1925 g. before the injection, and 1575 g. at the end.

In the peritoneal cavity was a small amount of turbid, reddish fluid. The liver was slightly enlarged, its capsule stretched. The color of the organ was dark red on the surface and grayish red on the cut surface. The central veins of acini were so strongly filled with blood that they seemed like dark spots on the cut surface. The kidneys were slightly enlarged and dark reddish in color. The spleen was normal in size but soft in consistency and reddish purple in color. In the thoracic cavity was a small amount of turbid, reddish fluid. The lungs showed a remarkable congestion. The heart was pale in color, the blood in both ventricles well coagulated. In the cranial cavity were also a few drops of turbid, reddish cerebrospinal fluid. No adherence was found between the brain surface an the inside wall of the cranium. Meninges showed dirty reddish in appearance. The cut surface of the brain was pinkish colored.

Histopathology: The liver sections showed remarkable capillary engorgement. Many glomeruli of the kidneys showed hyperaemia, and the tubuli were filled with coagulable substances. The spleen was also hyperaemic and its capsule was thickened. The vessels of the lungs were distended and filled with blood. There were also hemorrhages in places. The visceral pleura was thickened in infiltrated with leucocytes. A great number of injected spores were retained in the subarachnoidal spaces, and vivid phagocytosis of giant cells to spores were observed. (Fig. 3). There were seen the infiltration of lymphoid cells and a small amount of fibroblasts. (Plate II, 8 and 9). The superficial layer of the encephalon showed a cellular infiltration
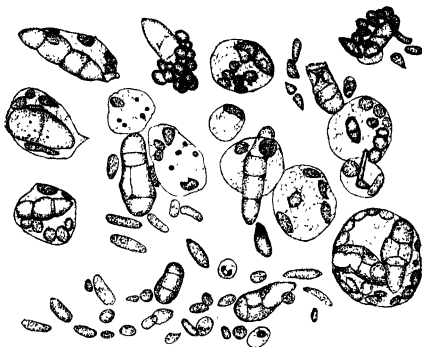
Fig 3. Phagocytosis of leucocytes to spores in the meninge

in places.

   Guinea pig 3. female. Injected subcutaneously with 0.5 cc. of the
spore suspension. Died 9 days after the injection. Body weights,
420 g. before the injection and 370 g. at the end.

   In the abdominal cavity were a few drops of reddish fluid. The
abdominal wall was moist. The liver was light brown in color, con-
siderably enlarged with thickened borders, and friable. The spleen
was a little enlarged, softer than normal, and dark reddish in color.
The kidneys were pale and oedematous. In the thoracic cavity, a
few drops of yellowish fluid were present. The lungs were dark red
in color, with increased consistency. A frothy red liquid was squeezed
from the alveoli. The heart was light brownish, and the blood was
well coagulated in both ventricles.

   Histopathology: The KUPFER's cells of the liver were swollen
and filled with brownished pigments. In the spleen was a prolifera-

tion of sinus endothelial cells in addition to a cellular infiltration in the splenic pulp. The malpighian corpuscles were somewhat obliterated. The kidneys showed some capillary engorgement. The glomeruli were slightly infiltrated with the leucocytes. The tubuli were somewhat swollen and contained a little coagulable substance. The septa of the alveoli of the lungs were thickened and engorged with blood. The air spaces of the alveoli were filled with accumulated exudate, made up in part of outwandered leucocytes and histiocytes and in part of desquamated epitherium.

Rabbit 4. male. Injected intravenously with 1.0 cc. of suspension. Killed 48 days after injection. Body weights, 1830 g. before the injection and 1985 g. at the end.

The noteworthy changes were seen only in the lungs. The lungs showed yellowish red in color. Microscopically was seen the thickening of the alveolar walls, and a red fluid in the alveolar lumens in which the histiocytes still remained.

Albino rat 8. female. Injected intraperitoneally with 0.5 cc. of the filtrate of bouillon culture. Died two days later. Body weights, 62 g. before the injection and 57 g. at the end.

In the abdominal cavity was a small amount of turbid, reddish fluid. The parietal peritoneum retained a few petechiae on the surface. The liver was enlarged, firm, and dark red in color. The kidneys were normal in size and pale reddish in color. In the thoracic cavity, a small amount of transudate was present. The lungs were dark red in color, and oedematous. The blood vessels of the surface of the brain were distended.

Histopathology : The liver showed capillary engorgement of blood. In the kidneys, glomerular hyperaemia were found. The lumen of the tubuli contained a lumpy and granular substance, staining well with eosin. In the spleen existed no remarkable changes. The lungs showed a remarkable hyperaemia. Hemorrphages were seen in places. The alveoli somewhat decreased their air spaces. The bronchial walls were infiltrated with leucocytes and the bronchial spaces contained some exudate. In the encephalon was very weak cellular infiltration.

The control animals, to which sterilized spores or the spores of an Alternaria species collected´from the leaves of the poppy were injected, showed no remarkable changes following such treatment.

Although I killed them for the purpose of comparison with the experimental animals in varying periods after the injection, only two representative cases will be described here.

Guinea pig 7. male. Injected intraperitoneally with 0.5 cc. of the suspension of sterilized spores. Killed 22 days later. Body weights, 620 g. before the injection and 640 g. at the end.

The macroscopical observation showed no remarkable changes.

Histopathology: The liver was slightly hyperaemic; the spleen was also rich in blood, and the nuclei of the splenic cells were generally vesicular. The kidneys were also somewhat congested and the tubuli contained a thread like substance. The lungs and brain were normal.

Albino rat 11. female. Injected intraperitoneously with 0.5 cc. of suspension of Alternaria sp. Killed 20 days after the injection. Body weights, 80 g. before the injection and 110 g. at the end.

Noteworthy changes occured only in the kidneys. They were dark red in color. The sections showed a considerable degree of capillary engorgement. The lumen of the tubuli contained some coagulated substance.

The autopsy and microscopical findings of all the animals injected with the spores showed simillar remarkable alternations: a) broncho or lobular inflammation of lungs, b) cellular infiltration of meninge, c) endotherial proliferation in splenic sinus and cellular infiltration of Malpighian bodies, and d) fibrinous or serous inflammation of kidneys. On the other hand, the pancreas and alimentary tracts showed no structural changes, and the changes in the heart were not remarkable.

These results lead us to conclude that the fungus studied by me is pathogenic to the usual laboratory animals. The affection is most severe in the lungs but that of the brain seems to be secondary. This is contrary to the relation noted in man, in which case the brain showed severe alteration while the lungs showed practically no alteration.

The pathogenicity of this fungus seems due, primarily to the large size of the spores, and secondarily to the toxin which is produced by the metabolism of the fungus, though I could not separate it from the cultures. Its presence, however, may be inferred from the non-pathogenicity when injected with sterilized spores. That the spore reacts as a foreign body and produces circulatory disturbances as its consequence may also be inferred from the fact that the animal inoculated

with sterilized spores also showed hyperaemia of several organs. The production of toxin by our fungus may be further inferred from the fact that the injection of an Alternaria sp. was not fatal to all the experimental animals, while the species found in the urine was always fatal to the animals, as was stated.

I regret that any satisfactory explanation as to the mechanism of spore excretion is not possible at present, and on this point a further investigation is necessary.

SUMMARY

1) A fungus found in the urine of a patient suffering from meningitis was studied. It seems that the fungus is identical to Alternaria tenuis Nees, from the cultural and pathogenic characters.

2) The very marked and definite changes in the lungs and other organs are considered sufficient evidence that the fungus studied by the author was pathogenic to the animal. The mortality rates were 67% in the guinea pig, 43% in the rabbits and 23% in the albino rats

3) The exotoxin production was examined. The intraperitoneous injection with 0.5 cc. of the filtrate of bouillon culture was fatal to mice and albino rats.

4) By the injection of sterilized culture of the fungus and living culture of Alternaria sp. collected from leaves of the poppy, no remarkable disturbance was observed, except the slight congestion of various organs. The feeding investigation with spores caused no remarkable disturbance, either.

5) Many spores were found in the urine, lungs, spleen, liver, gall-bladder and brain, in varying periods after the intraperitoneal and subcutaneous injections. The number of these spores was reduced by the lapse of time, and the detection of spores after one month was usually negative in any organ.

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of Gomphrena Globosa. L Bull of Col. of Agri and Forest Morioka no. 9.

EXPLANATION OF PLATES

PLATE I

1. Conidia produced on the persimmon agar
2-5. Showing the conidia formation on the tip of conidiophore
6. Hyphae and vesicular, highly vacuolated and thin walled bodies

PLATE II.

7. Colony formed on the SABOURAUD's glucose agar plate (8 days old culture
×2/3)

8 Meninx of intracranially injected rabbit, showing the presence of spores
and cellular infiltration (×57).

9. Highly magnified figure of a part of fig. 8. (×480).

10. Desquamated epitherial cells of lung of rabbit following the intravenuos
injection of spores (×480)

PLATE III

11. Cut surface of lung of intravenously injected rabbit.

12. Circumscript pleuritis of same animal (×30).

13-14. Insular necrosis of lung of the same. (×57).

15. Highly magnified figure of a part, of fig 14 showing the presence of a
spore which is captured in a giant cells (×480),
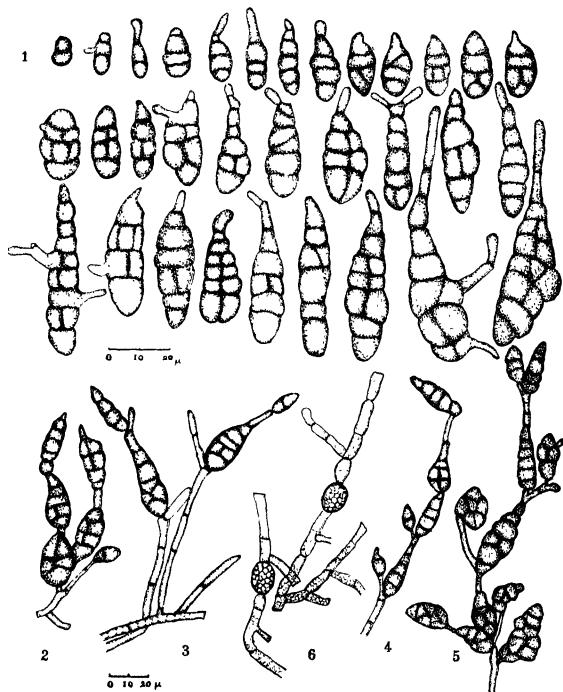
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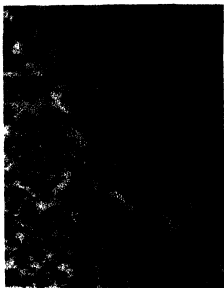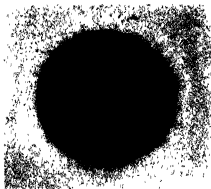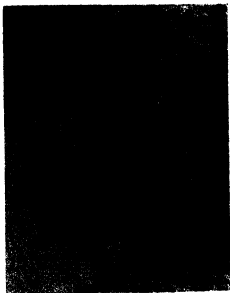
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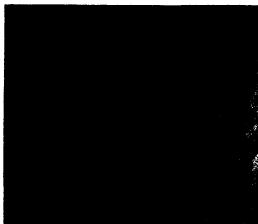
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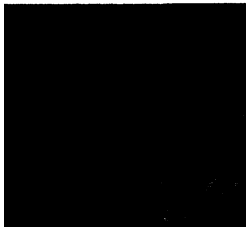
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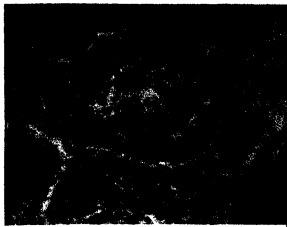
T. OHUYE: Fungus found in a Patient, Meningitis.

On the Sexual Differences in the Newt, Diemictylus pyrrhogaster (Boie).

By

TADAO UEKI

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(With 1 text figure)

The external characters of the newt were already observed and described by T IWAKAWA (1889) and L STEJNEGER (1907), but much remains to be studied about this interesting Anuran species The object of the present study is to observe the sexual dimorphism in relation to colour marking together with the various measurements of the body its component internal organs and their water content, and the range of the fluctuation of the body weight

MATERIAL USED

Diemictylus pyrrhogaster is a species of the newt widely distributed in Japan and is commonly found in old ponds, swamps, and road side drains The newt is easily collected with a net because of its slow movement The most of those which I used in my experiments were collected in a little pond at Futatsusawa in the vicinity of Sendai, and at Bagyunuma in the vicinity of Shiroishi, Miyagi Prefecture, a carp cultural pond The number of newts collected at these two ponds are respectively as follows

	♂	♀
At Futatsusawa	71	51
At Shiroishi	354,	459
Total	425,	510

Namely, altogether 935 newts were utilized for this study The newts collected in different ponds were used for different experiments, and in no instances the newts from different sources were mixed for one set of the experiments Those newts which were not used at once were kept in a fish pond and fed with Tubifex and Limnodrilus.

The details concerning the time when collected, and when used are shown in Table I.

<div align="center">TABLE I.</div>

Date on the newts from corresponding ponds are found in Tables.	II, III, V, VIII.	IV, V.	VI, VII, IX.
Locality and time of collection.	Futatsusawa, on July 1st, 1927	Futatsusawa, on November 10th, 1927.	Shiroishi on November 23rd, 1927.
Feeding.	Limnodrilus and Tubifex, every morning.	Limnodrilus and Tubifex, every morning.	Kept in tap-water without food.
Examined.	July 1st — July 12th, 1927	November 11th — November 30th, 1927	November 25th — December 1st, 1927.
Number of newts.	40(20 ♂ +20 ♀.)	82(51 ♂ +31 ♀.)	813(354 ♂ +459 ♀.)

The animals were killed with chloroform and both the gross body weight and gross volume, together with the lengths of the total as well as of component parts of the body were recorded. The very general distribution of color markings viewed from the side was sketched from some newts. The head (from tip of snout to gular fold) and the tail (from anterior angle of vent to tip of tail) were then removed, and the blood allowed to escape, and separately weighed. The weight of the trunk with limbs was estimated by subtracting the weight of the head and tail from the entire body weight. The weight of blood in both head and tail is thus added to the weight of the trunk, but fortunately the amount of error from such treatment is insignificant, owing to the very minute quantity of blood in these two regions just stated. Finally various' organs were taken out of the trunk separately by cutting the ventral wall, not along the middle line, but slightly to the side in order to avoid the sectioning of the abdominal vein. All the organs under discussion can easily be isolated from their surrounding structures. The organs which were removed and weighed are as follows; brain, eyeballs, heart, thyroid, spleen, pancreas, stomach, intestines, liver, gall-bladder, lungs, kidneys, ovaries, oviducts, testis, cloacal glands, and fat bodies.

It required nearly one and a half hours for removal as well as

for weighing to 0.001 gram all those organs from one newt, The
net body weight was obtained from the gross body weight by sub-
tracting the weights of the contents of stomach and intestines and all
parasites which were found in various organs. In order to avoid the
error due to evaporation during removal of the various organs, I placed
a number of wet clothes near by and began with the minute organs.

SECONDARY SEXUAL CHARACTERS.

At the young stage of the newt the secondary sexual characters
are not distinct, but with those body weight reached to about 3 or
4 grams the differences are easily noticeable. Some of the prominent
differences according to sex (noticed from the fully adult newts, unless
otherwise mentioned) are as follows:
1. The female is larger than the male. From the measurements
made on about 800 adult newts, it was found that the total body
length of the adult female measures about 8 to 13 cm., while that
of the male ranges from 7 to 11 cm. (tail-fin included) ; and the body
weight of the full grown female is about 10 grams., while that of the
male is 6 grams. The difference seems to be the result partly of the
heavier weights of gonads and cloacal glands. According to Table
IV, the average value of the body weight being equal, that is, 5.3 gram,
ovaries plus oviducts are 0.46 grams + 0.37 grams = 0.83 grams, while
testes plus cloacal glands are 0.14 grams + 0.17 grams = 0.31 grams, so
that the percentage will be 16% in the female while 6% in the male.
2. At puberty, both the flap-like patroid glands and the lateral
glandular ridges (dorso-ventral and ventro-lateral of the trunk) are
larger in male than in the female, and the scapular glands develop
only in the male. (See L. STEJNEGER: Herpetology of Japan and
Adjacent Territory. pp. 16-21.)
3. The vent of the male is surrounded by a globular swelling,
the surface of which is coarsely pustular, and the opening exhibits a
longitudinal slit, but in the female vent is placed on an oval swelling
which is elevated from a compressed base and the opening itself is
circular in shape.
Professor S. HŌZAWA called my attention to the fact that in the
sexually mature male, long radiating papillae with many hair-like process

develop inside the vent temporarily from April to May.

    4. The fore legs and the hind legs of the male are relatively longer than those of the female, and the longest third toe of the male measures about 6.5 mm., while that of the female measures 5 mm.

    5. The tail of the male differs from that of the female in several respects. It is strongly compressed dorso-ventrally and along the middle lines on both surfaces (above and below) are found the fins. The heights of these are equal, and they suddenly taper near the end of the tail, while in the female the fins are totally absent from the tail and the tail as a whole tapers gradually to the end.

    The relation between the tail length and the height of the tail at the middle is as follows:

Average of 40 newts (20 ♂, 20 ♀.).

		♂	♀
Absolute length in mm.	Tail length	49.2	57.5
	Tail height	7.2	6.9
Percentage length	Tail length	100.0	100.0
	Tail heigth	14.7	12.0

    In the female the ventral 1/5 of the tail fin exhibits a deep blood red color, while in the male the red color is not only very weak but it fades away dorsally without forming any boundary line between the ventral red and dorsal dark.

    The extention of usual red coloration of the ventral suface varies according to the individuals, irrespective of the sex.

    6. In the sexually mature male, the body surface is very soft to the touch, and the coloration of the body is very beautiful, exhibiting a complex arrangement of colors, — blackish green, lighter green, steel blue, blood red, etc.

SEXUAL DIFFERENCES IN MEASUREMENT OF WEIGHTS AND DIMENSIONS.

A. WEIGHTS OF INTERNAL ORGANS.

    In Table II are given the values of the observed body weights and all the organs examined, based on 40 newts (20 ♂, 20 ♀.) which

TABLE II.

		Homo sapiens (after Welcker, H.)		Salamandra maculosa (After Welcker, H.)		Diemictylus pyrrhogaster	
	Species	Male	Female	Male	Female	Male	Female
	Sex						
	Numbers	5	4	1	1	20	20
	Net body weight	57.5 Kilograms	51.2 Kilograms	12.1	22.3	4.1	6.1
Absolute weight in grams	Brain	1461	1249	.031	.044	.018	.021
	Eyeballs	12	12	.071	.085	.019	.020
	Heart	374	333	.053	.048	.006	.010
	Thyroids					.0006	.0011
	Spleen	149	111	.038	.072	.017	.027
	Pancreas	86	72	.025	.048	.008	.013
	Alimentary tract	2197	1426	.644	1.560	.159	.242
	Liver	1754	1342	.521	2.258	.226	.334
	Gall-bladder					.007	.012
	Lungs	1471	774	.085	.130	.017	.025
	Kidneys					.037	.041
	Ovaries						.519
	Oviducts						.219
	Testes					.234	
	Cloacal glands					.027	
	Fat body					.179	.129
	Net body weight	100.00	100.00	100.00	100.00	100.00	100.00
Percentage weight	Brain	2.57	3.61	.26	.19	.45	.34
	Eyeballs	.12	.18	.59	.38	.46	.33
	Heart	.66	1.27	.44	.22	.14	.16
	Thyroids					.014	.018
	Spleen	.26	.27	.32	.32	.42	.44
	Pancreas	.15	.14	.21	.22	.19	.21
	Alimentary tract	2.56	3.76	5.34	7.00	3.87	3.97
	Liver	3.09	4.10	4.32	10.13	5.52	5.48
	Gall-bladder					.16	.20
	Lungs	2.59	1.34	.71	.58	.41	.41
	Kidneys					.89	.68
	Ovaries						8.53
	Oviducts						3.60
	Testes					5.72	
	Cloacal glands					.67	
	Fat body					4.88	2.12

were collected during the month of July from Futatsusawa.

In this table are included also the percentage weight, and for comparison, the data on Homo sapiens and Salamandra maculosa. I have already mentioned that the total body weight of the female is much heavier than that of the male and this relation is clearly shown in this table.

The sexual differences shown by each organ examined are better shown when the percentage values are examined, that is, the weight of brain, eyeballs and kidneys of the male surpass the female, but, on the contrary, the alimentary tract and gall-bladder are relatively heavier in the female than in the male. In general, the same phenomena occur with Salamandra maculosa, as will be seen from Table II, while in Homo sapiens the relative weights of brain and eyeballs are smaller in the male than in the female, although in the case of the alimentary tract the same relation is shown as in the case of the newt.

B   VARIOUS OTHER MEASUREMENTS.

In Table III are given the values of the observed body lengths and the lengths of various parts examined. In this table are also

TABLE III.

Sex	Absolute length in mm.		Percentage length	
	Male	Female	Male	Female
Numbers	20 newts from 3.2 to 5.1 grams	20 newts from 4.3 to 8.2 grams		
Total body length	93	111	100	100
Head: From tip of snout to gular fold	12	14	13	13
Trunk· From gular fold to anterior angle of vent	32	40	34	36
Tail: From anterior angle of vent to tip of tail	49	58	53	52
Width of head	10	11	11	10
Fore leg	19	20	20	18
Hind leg	20	22	22	19
Longest toe (the third toe)	6.0	5.6	6.5	5.0
Height of tail in middle	7.2	6.9	7.8	6.2

included the percentage values of the same data just given. In general, the absolute body length of the female is longer than that of the male, and a similar relation holds true in all other parts which were measured. On the other hand, when these absolute values are transformed into the percentage values then the relative values of all
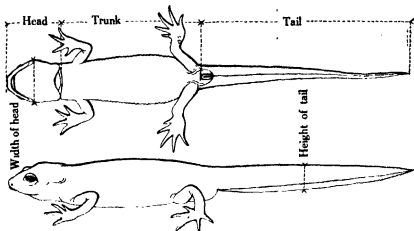
Fig 1

the measurements shown an opposite relation from the above. I have given the two diagramatical illustrations showing various parts of the body discussed in this paper (Fig. 1).

C. GROWTH OF PARTS OF THE BODY AND VARIOUS ORGANS WITH RESPECT TO THE ENTIRE BODY WEIGHT.

In order to show the relations just stated more clearly I have plotted these values, according to the increasing body weight, in Chart I, Figs. 1–14, based on the data given in Table IV. To the body weight is included the weight of gonads. I have already shown various weight and length relations which are given by the adult newts when compared with Salamandra maculosa and Homo sapiens. In this section it is intented to study the growth of parts of the body and various organs with respect to the entire body weight, based on 82 newts (51 ♂, 31 ♀.) which were collected, also from Futatsusawa,

TABLE IV.

Body weight	No.	Head	Trunk	Tail	Brain	Eyeballs	Heart	Spleen	Pancreas	Stomach	Intestines	Liver	Lungs	Kidneys	Fat body	Ovaries	Oviducts	Testes	Cloacal glands
Male																			
3.0	1—	.46	2.3	.35	.019	.019	.005	.011	.006	.04	.05	.18	.009	.009	.027			.08	.07
3.1/4		.51	2.6	.38	.020	.020	.006	.014	.007	.05	.06	.22	.010	.044	.024			.09	.09
4.0		.57	3.0	.41	.023	.022	.007	.016	.008	.06	.06	.25	.012	.053	.036			.11	.11
4.5		.60	3.4	.46	.026	.025	.008	.018	.009	.07	.07	.29	.013	.058	.095			.12	.14
5.4		.77	4.3	.60	.027	.027	.010	.018	.010	.08	.07	.33	.014	.060	.070			.13	.18
5.4		.83	4.7	.55	.030	.033	.010	.019	.011	.08	.08	.38	.016	.070	.044			.15	.18
6.2		.85	5.0	.69	.033	.033	.011	.018	.013	.09	.08	.42	.016	.079	.066			.16	.19
6.6		.88	5.0	.72	.034	.034	.011	.018	.013	.09	.08	.46	.017	.084	.053			.16	.20
Average 4.8		.67	3.6	.48	.025	.027	.008	.017	.0.9	.07	.07	.31	.013	.062	.038			.12	.14
Female																			
4.2		.67	3.2	.43	.021	.020	.007	.016	.009	.06	.07	.23	.014	.025	.039	.34	.27		
4.5		.60	3.4	.51	.020	.020	.008	.018	.011	.06	.08	.24	.015	.029	.024	.37	.37		
5.0		.66	3.8	.57	.022	.021	.009	.018	.011	.09	.08	.29	.016	.031	.025	.43	.34		
6.6		.72	4.3	.56	.028	.022	.010	.021	.013	.09	.10	.36	.018	.033	.029	.49	.39		
6.5		.78	4.6	.64	.032	.024	.011	.022	.014	.11	.11	.41	.019	.037	.040	.56	.41		
6.5		.82	5.0	.60	.030	.024	.011	.022	.015	.11	.11	.46	.020	.041	.046	.66	.46		
7.0		.89	5.4	.67	.036	.031	.012	.022	.016	.12	.14	.46	.021	.048	.061	.61	.62		
8.4		1.08	6.5	.77	.041	.031	.013	.022	.018	.12	.14	.61	.024	.049	.029	.74	.89		
8.4		1.13	7.7	.88	.034	.034	.014	.026	.020	.14	.14	.61	.027	.057	.072			.19	.14
Average 6.4		.80	4.9	.61	.025	.025	.010	.020	.014	.10	.10	.39	.019	.037	.032	.56	.46	.14	.17
Average (4.0—6.6 ♂) 5.2		.73	4.0	.52	.029	.028	.009	.018	.012	.08	.07	.34	.015	.068	.041				
Average (4.2—6.6 ♀) 5.2		.69	4.1	.54	.028	.023	.009	.019	.012	.09	.09	.30	.017	.034	.032	.46	.37		
Percentage differences		+5.6—3.6	—3.6—3.8	+21.4	+21.4	+24.1	1.0—5.6	1.0—5.6	+0.15	—12.5	—12.5	.31	—13.3	—13.3	+52.9+21.9				

during the month of November. The former data given in Table II are not included in this study but those of the adult are compared to each other in Table V.

Since the relations between the two sexes concerning various measurements are clearly shown in both Table IV and Chart I, I shall omit detailed description and will limit myself to those measurements of which the relation is not expressly shown in the table or in the chart.
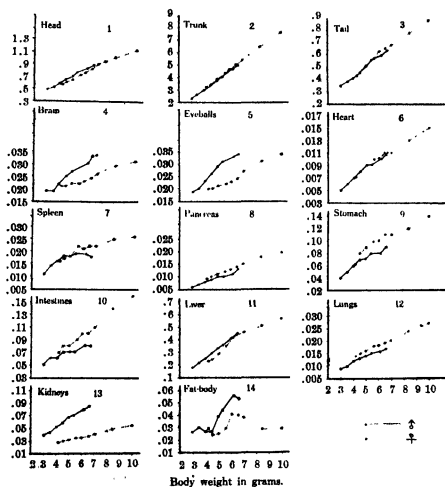
Chart I.

TABLE V.

	Locality of collection	Futatsusawa		Futatsusawa	
	Examined	July 1st — July 12th, 1927.		November 11th — November 30th, 1927.	
	Sex	Male	Female	Male	Female
	Number of newts	20 newts from 3.2 to 5.1 grams	20 newts from 4.3 to 8.2 grams	43 newts from 3.1 to 5.4 grams	25 newts from 4.5 to 6.1 grams
Absolute weight in grams	Net body weight	4.1	6.2	4.2	6.1
	Brain	.018	.021	.023	.024
	Eyeballs	.019	.020	.025	.024
	Heart	.006	.010	.007	.010
	Spleen	.017	.027	.016	.021
	Pancreas	.008	.013	.008	.013
	Stomach	.056	.092	.060	.101
	Intestines	.103	.150	.060	.100
	Liver	.226	.334	.271	.387
	Lungs	.017	.025	.012	.019
	Kidneys	.037	.041	.055	.036
	Fat body	.179	.129	.022	.032
	Ovaries		.519		.534
	Oviducts		.219		.440
	Testes	.234		.111	
	Cloacal glands	.027		.128	
Percentage weight	Net body weight	100.00	100.00	100.00	100.00
	Brain	.45	.34	.55	.59
	Eyeballs	.46	.38	.59	.39
	Heart	.14	.16	.17	.16
	Spleen	.42	.44	.38	.34
	Pancreas	.19	.21	.19	.21
	Stomach	1.37	1.50	1.42	1.66
	Intestines	2.50	2.47	1.42	1.64
	Liver	5.52	5.48	6.45	6.34
	Lungs	.40	.41	.29	.31
	Kidneys	.89	.68	1.31	.59
	Fat body	4.38	2.12	.76	.52
	Ovaries		8.53		8.75
	Oviducts		3.60		7.21
	Testes	5.72		2.63	
	Cloacal glands	.67		3.05	

Head. — The male head is a little heavier than the female head. This difference seems to be resulted partly from the heavier weights of brain and eyeballs in the male than of those in the female (Chart I, 1).

Trunk. — In the weight of trunk, the difference between the sexes in negligibly small being only $+2.5\%$ in favour of the female (Chart I, 2).

Tail. — In this character, the difference is also slight being $+3.8\%$ in favour of the female (Chart I, 3).

Brain. — Here the sex difference is very clear, being as much as $+21.4\%$ in favour of the male (Chart I, 4).

In Rana nigromaculata, KOMINE (1924) also found differences in the brain weight according to sex and the male showed relatively heavier brain weight than the female. It is curious to note that, in Homo sapiens, the female brain is relatively heavier than the male brain (WELCKER, H.).

Eyeballs. — The male has the heavier eyeballs than the female, being $+24.1\%$ in favour of the former (Chart I, 5).

It is interesting to note that the weights of the brain and eyeballs are heavier in the male than in the female.

Heart. — The sex difference is least in the heart weight (Chart I, 6).

Spleen. — In the case of the spleen, the sex difference is clear and the curves of both male and female continue to rise upwards gradually, though a slight fall is noticed toward the end (Chart I, 7).

Pancreas. — The sex difference is not so great as that shown by the central nervous system, and the female possesses a relatively heavier pancreas (Chart I, 8).

S. HATAI (1918) noted the same sexual relation in the albino rat, though W. E. KELLIKOTT (1908) failed to find it in the dog fish. WELCKER's data (1903) reveal the existence of a similar sexual difference in several of the lower vertebrates, such as Rana, Triton, Salamandra etc.

Stomach. — The female newt possesses a heavier stomach, though in the albino rat HATAI (1918) found no sex difference.

Intestines. — The sex difference is clearly marked in the weight of intestines as with the stomach, the difference being as hight as
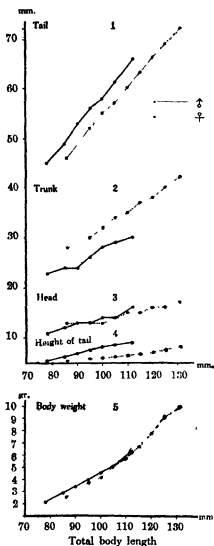
Chart II.

Tail — Anterior angle of vent to tip of tail,
    in mm.
Trunk — Gular fold to anterior angle of vent,
    in mm.
Head — Tip of snout to gular fold, in mm.
Height of tail — Height of tail in middle, in
    mm.
Body weight — Gross body weight, in grams.

+28.6% in favour of the female (Chart I, 10).

Liver. — The liver of the male is a little heavier than that of the female (Chart I, 12).

Kidney. — The kidney weight presents great difference according to sex, the difference being as hight as +52.9% in favour of the male (Chart I, 13).

Fat body. — The sex difference is distinct and the male's is heavier than the femal's (Chart I, 14).

Ovaries and Oviducts. — The primary sex organs in the female increase as the body weight increases.

Testes and Cloacal gland. — These organs, possessed by the male sex alone, increase as the body weight increases.

D. GROWTH OF COMPONENT PARTS OF THE BODY IN RELATION TO THE INCREASING BODY LENGTH.

In Chart II — Fig. 1–4 are given the measurements of component parts (head, trunk and tail)' of the body, in all of which the sex differences are clearly shown. The data are given in Tables VI and VII.

TALLE VI.

Number of individuals studied (Male)	Total length, in mm.	Tip of snout to gular fold, in mm.	Gular fold to anterior angle of vent, in mm.	Anterior angle of vent to tip of tail, in mm.	Height of tail in middle, in mm.	Gross body weight, in grams.
7	78	11	23	45	5.5	2.2
45	85	12	24	49	6.4	2.9
79	90	13	24	53	6.9	3.4
123	95	13	26	56	7.6	4.0
74	100	14	28	58	8.0	4.6
21	105	14	29	62	8.5	5.1
5	112	16	30	66	9.0	6.3
Total 354(♂)						

TABLE VII.

Number of individuals studied (Female)	Total length, in mm.	Tip of snout to gular fold, in mm.	Gular fold to anterior angle of vent, in mm.	Anterior angle of vent to tip of tail, in mm.	Height of tail in middle, in mm	Gross body weight, in grams
4	86	13	28	46	5.5	2.6
25	95	13	30	52	5.8	3.8
57	100	13	32	55	5.9	4.2
63	105	14	34	57	6.2	5.2
93	110	15	35	60	6.5	5.8
86	115	15	37	63	6.7	6.7
84	120	16	38	66	7.3	7.8
48	125	16	40	69	7.7	9.1
19	131	17	42	72	8.1	9.9
Total 459(♀)						

The length of the head (the measurement taken from tip of snout to gular fold is called the head length) tends to be longer in the male, though the difference between the sexes is very slight.

The length of the trunk (from gular fold to anterior angle of

vent) of the female is, however, distinctly longer than that of the
male.

   The length of the tail (from anterior angle of vent to tip of tail)
and its width at its middle region are distinctly greater in the male.

   The tail is generally longer than the sum of the length of the
head and the trunk in both sexes

       E BODY WEIGHT IN RELATION TO THE BODY LENGTH

   It is clear that for any given body length the male gives greater
body weight, and vice versa

   What was found true of the newt is opposite to the facts known
of the albino rat, found by DONALDSON and HATAI (1911), in which
the body weight of the male is less than that of the female for the
same given body length The data of measurement are given in
Tables VI and VII and in Chart II, Fig. 5.

       VOLUME, SPECIFIC GRAVITY, AND WATER CONTENT
                     OF TOTAL BODY

   The volume of the body was measured by displacement of water
by placing the newt in a long narrow measuring cylinder, and the
specific gravity was obtained from the relation $\frac{\text{Body weight}}{\text{Body volume}}$.

   The water content of the entire body was determined by placing

                        TABLE   VIII.

Sex	Male	Female
Number of newts	20	20
Body weight, in grams	4.09	6.09
Body volume, in CC	3.90	5.88
Specific gravity	1.05	1.04
Water content, in % o (Materials from Shiroishi)	76.7	78.7

the newt in a weighing bottle after the ventral and dorsal body walls
were cut open at a temperature of 95°-105°C for two weeks (Table VIII).

FLUCTUATION OF BODY WEIGHTS OF ADULT NEWTS.

A large number of adult newts was collected in the Bagyunuma and the fluctuation of their body weights were determined. 300 males and 300 females were picked at random for this purpose.

The frequency distribution of the body weights are given in Table IX. As will be seen from the table, the male body weights range from 1-2 grams to 6-7 grams while those of the female range from 2-3 grams to 11-12 grams. Thus, considerable difference is shown in the range of variability between the two sexes. The wider range of body weights in the females is due to their considerably larger body size, compared with the males. This is the same result that SASAKI (1926) found in Carassius auratus.

TABLE IX.

Weight in grams	Number of individuals	
	Male	Female
1— 2	1	
2— 3	33	4
3— 4	128	15
4— 5	105	37
5— 6	28	64
6— 7	5	54
7— 8		52
8— 9		31
9—10		25
10—11		12
11—12		6
Total	300 (♂)	300 (♀)

The mean value of male newts was found to be 3.97 grams while that of females was found to be 6.72 grams.

SUMMARY.

In the following, the more important facts are summarized:

1. The male newts are far smaller than the female; the mean body weight of the male is 3.97 grams against 6.72 grams for the female.

2. In the males, the patroid glands and lateral glandular ridges develop especially during the sexually active period, and the vent not only swells but also many hair-like processes grow temporarily on its inside.

3. In the sexual season the male is beautifully multicolored,

codtrary to the simple black or brown-black color of the female.

At this period the skin of the male becomes soft and velvety and the sex can be distinguished by mere touch with the fingers.

In the male, less than one-fifth of the entire length of the tail is blood-red colored.

4. The length of head, tail, legs and longest toe, and the width of the head, and the height of the tail are comparatively longer in the male, but the trunk is longer in the female.

5. In relation to body weight, the weights of head, brain, eye-balls, liver, kidneys, and fat body are heavier in the male, but that of stomach, pancreas and lungs are heavier in the female.

6. For any given body length, the body weight of the male is heavier than that of the female.

7. The range of the fluctuation of the body weight is wider in the female than in the male.

8. The water content of the female body tends to be higher and its specific gravity lower than those of the male.

GENERAL REMARKS

The aim of this study was to trace the sex differences in the newt. It is noticeable that there are the cloacal glands in the male, peculiar to this sex, and that besides there is the male gonad in the male as the primary sexual character. Side by side with the observation of the secondary sexual characters, I also made a special investigation of various measurements of the body and its component internal organs. The latter investigation was done in Summer and Autumn only, but I think it necessary to further extend the investigation through Winter and Spring, and carefully study and compare their seasonal changes throughout the whole year. I believe the sex differences in animals of various zoological orders will be determined not only by the differences of their so-called secondary sexual characters and various measurements both external and internal, but also by their habits peculiar to each sex, and by macroscopical, microscopical, and biometrical study of the viscera, skeleton, muscles, circulatory system, nervous system, etc. As a result of statistical observations on Rana and Hyla, KAHN (1900) came to the conclusion that the

expansible "Inscriptions elasticae" are characterestic only of the male in the general adult frogs; while, F. A. E. CREW (1920), after his observation of the differences of poise in Rigor mortis, found that there are various sex differences, especially in their sexual season. If, in like manner, investigations were made on the subjects from various points of view, many unexpectedly interesting results would be obtained, though at present the researches generally followed are those of so-called secondary sexual characters.

Now, in young immature newts, the secondary sexual characters are not yet developed, but in the adult newt these characters are distinct, and especially in their sexual season these appear most distinct as is the case in other animals. These characters are well developed in Arthropoda, but, putting them aside for the present, I wish to remark a little on the five classes in Vertebrates.

In Pisces, well developed secondary sexual characters are seen in Xyphophorus, Cynolebians, Misgurnus, Duymaeria and Helichoeres, but in general, this character is less remarkable. J. T. CUNNINGHAM (1900) gives a long detailed list of species in the animal kingdom in which the dimorphism is present but give very few cases about Pisces. In Amphibia, few species of Urodela or Anura show development of secondary sexual characters. The same is true with the cases in Reptilia. However, in Aves also in Mammalia we notice well developed secondary sexual characters in most species.

An intimate relation between the secondary sexual characters and the hormones is believed to exist by numerous recent investigators. MEISENHEIMER (1911) in Anura, and ARON (1924) in Urodela, found that the secondary sexual characters disappeared after the removal of the testis, and numerous interesting facts demonstrated by STEINACH on the relation between the removal of primary sex glands and the secondary sexual characters are too well known to be repeated here. J. OYAMA (1923) measured the sizes of various endocrine organs of the newt, though the weights were not determined. If the observations were further extended to determine the relation between the secondary sexual characters and the size of the endycrine organs, it might add further important information to the physiology of these organs, and at the same time throw some light on the true significance of the so-called secondary sexual characters. OORDT (1925) found in

Xyphophorus Helleri Heckel the successive development of the secondary
sexual characters in association with the successive development of
Testicular tissues. ARON (1924) also found in five species of Urodela
that the degree of development of secondary sexual characters is
directly proportional to the increase of glandular tissue in the testis.
It would be interesting to determine whether or not in Anura the
secondary sexual character is better developed in the species which
possesses the relatively larger and better grown endocrine organs.

I wish to express my gratitude to Prof. S. HATAI, at whose sug-
gestion this work was undertaken. for encouragement given throughout
the entire course of the work. I also wish to thank Prof. S. HŌZAWA
and Mr. K. SASAKI for much valuable advice.

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On the Physiological Axial Gradients of Chaetopod Annelids.

I. Types of Axial Gradients Examined by Onset Temperature of Heat Shortening.[1]

By

Yisamu Watanabe.

(With 34 Textfigures)

INTRODUCTION.

In axiate organisms the activity of physiological functions or even the chemical composition of the body shows a graded variation along the body axis, that is to say, the existence of CHILD's so-called *Physiological Axial Gradients* is evident as has been already clearly demonstrated in many species of organisms.[2] According to CHILD the physiological axial gradient is the simplest expression of a general organismic pattern, both in functions and structures, of the organism as a whole and therefore, the form of such gradients shown by organisms in natural or normal environmental conditions is specific and characteristic for each species of organisms. However such gradients are subject to change within certain limits under some artificial mechanical operations or chemical treatments, or they are transformed as the results of the necessary modifications of organismic pattern during the course of normal development.[3]

Hence, the similarities, variations or other comparable relations, among these gradient forms in different organisms and especially in organisms systematically nearly related, whether they are of adult, or embryonal, or larval stages, might imply some important biological

[1] A contribution of the Marine, Biological Station, Asamushi, Aomori-Ken. No. 51
[2] CHILD's book (1924) Chapters VII, VIII, IX, X and literature cited there
[3] CHILD's books (1915 a, b, 1921, 1924), and a brief statement of the important conclusions drawn from his thoughts and experiments is also described in his own epitom "Physiological Gradients" in Protoplasma Bd V (1928)

significance, and therefore in the present series of investigations I
attempted to search for such relations of physiological axial gradients
in some common Japanese chaetopod annelids.

Concerning the physiological axial gradients of chaetopods, the
observations of L. H. HYMAN and her collaborators[1], on the suscepti-
bility to cyanides, oxygen consumption rate, electrical polarity and
other physiological properties, indicated that the gradients of worms
belonging to this group were either monopolar or bipolar, that is, the
physiological activities were highest at the head region and decreased
gradually towards the tail region along the axis of the body, or were
high at the head region and lowest at about the middle region, in-
creasing again posteriorly The work of HATAI (1924) on the heat-
shortening gradient and that of the content of water, and my previous
work (1928) on the electrical polarity ʾn the earthworm, Perichaeta
(= Pheretima), also showed that the gradients of this worm were of
bipolar form.

However, all these works were based only on several oligochaetes
and a few species of Nereis and others, so that it is premature to
conclude that the typical annelid gradient is of monopolar or bipolar
form alone, unless several other representative species of this group
are examined. Last summer (1928) I had an opportunity to investigate
the axial gradient of heat shortening in the lugworm, Arenicola cristata,
and detected that the gradient of this worm was neither monopolar,
nor bipolar, but that it formed a quite different shape from those of
the above two ; the onset temperature of heat shortening was highest
at about the middle part and decreased towards both extremities of
the body along the anteroposterior axis, though the anal segments
were again higher than the intervening part. Thus the gradient form
resembles the sine-curve shown between ʾangles of 0° and 360°. (See
Chart 13). As the result of such an investigation I could not help
suspecting that there were several types of gradients in annelids other
than the monopolar and bipolar types.

Herewith, the present research was undertaken with the hope of
presenting the data obtained from examinations of heat shortening

[1] HYMAN, L. H. (1916); HYMAN, L. H. and A. E. GALIGHER (1921); HYMAN, L. H.
    and A. W. BELLAMY (1922).

concerning the axial gradients in groups of chaetopods covering different species, and of classifying them into some possible groups according to the difference in their gradient forms.

MATERIALS[1] AND METHODS.

The following species have been employed in this experiments:
Polychaetes,
    Nereidae (Lycoridae)
        (1) *Nereis mictodonta* MARENZELLER
    Aphroditidae
        (2) *Polynoë vexillaria* (MOOR)
        (3) *Polynoë* n. sp. ?
    Eunicidae
        (4) *Marphysa iwamushi* IZUKA
    Nephthydidae
        (5) *Nephthys caeca* MÜLLER
    Glyceridae
        (6) *Glycera* n. sp. ?
    Cirratulidae
        (7) *Cirratulus dasylophius* MARENZELLER
    Terebellidae
        (8) *Terebella* sp. (*T. debilis* ?)
    Chlorhamidae
        (9) *Stylaroides plumosa* MÜLLER
    Arenicolidae
        (10) *Arenicola cristata* STIMPSON
    Maldanidae
        (11) *Praxillella* n. sp. ?
    Sabellidae
        (12) *Potamilla myrops* MARENZELLER
    Eriographidae
        (13) *Myxicola infundibulum* MONTAGUE
Oligochaetes,
    Tubificidae

[1] Most of the polychaetes used here were identified by Dr A. IZUKA and *Allolobophora calignosa* (SAV.), by Mr. S. SEKIGUCHI.

        (14) *Branchiura* sp.
    Perichaetidae
        (15) *Pheretima communissima* (GOTO et HATAI)
    Lumbricidae
        (16) *Allolobophora foetida* (SAVIGNY).
        (17) *Allolobophora caliginosa* (SAVIGNY)
    These species, for the most part, were collected and examined at
the Asamushi Marine Biological Station, Aomori-Ken, but some poly-
chaetes collected from the coast of Seto-Uchi or Seto Inland Sea were
examined at the Ōchō Fish-Cultural Station of the Imperial Fishery
Institute, Ōchō-Mura, Ōsaki-Simojima Island, Hiroshima-Ken.
    The worms used as material were all of adult form and were as
nearly the same in body length as possible, but in the cases of some
species of which I could not collect easily enough worms to select
freely those of proper length only, the body length of worms was
somewhat diverse. And with strict attention, I selected the worms
without any trace of regeneration and without even any injury, for
the temperature of heat shortening, I supposed, varied with the degree
of regeneration. Variations of gradients due to sexual difference were
also tested in some species (*Marphysa iwamusi* and *Arenicola cristata*),
but from the preliminary test it did not appear to affect conspicuously
the present consideration, so that I did not try such detail examinations
in each species. (See Charts 6, 13.) In most cases of large specimens
the body wall alone, i. e., what contained chiefly integuments and
muscular tissues, was used; but when the worms were too small or
too fragile to make possible the separation of the body wall from the
other parts of the body, entire pieces of the specimen including the
alimentary canal and other internal organs were used. Even in such
cases the result of the experiment seemed to be quite, though not
perfectly, reliable in regard to the present purpose, for most of the
contractile or muscular tissues of chaetopods were represented by those
in the body wall. A piece of body or body wall used, in the examina-
tion was cut into a strip of a convenient length along the longitudinal
axis of the body. The length of the strips from the different parts
of the worm body was as nearly as possible the same when they were
tissues belonging to the same species of worm.
    C. W. LATIMER (1898), H. M. VERNON (1899) and recently G. D.

SHAFER (1928 a, b) showed that fatigued muscles shortened less and a lower onset temperature than normal muscles in heat shortening, and that completely fatigued muscles did not show heat shortening; and HATAI (1924) also pointed out that to employ fresh and active specimens was essential to the precise determination of onset point of heat shortening on gradual heating, since older and less active specimens shortened too gradually to determine this point of onset with accuracy. In all cases of experiments, I took these indications into my consideration and supplied the material as freshly as possible.

The method of measurement of the onset temperature of heat shortening was very simple and followed HATAI's method (1922) with some slight modifications. By means of simple suspension method the shortening of a piece of tissue was made to trace on the slowly revolving drum of a kymograph on which, at the same time, the temperature was marked every one-tenth degree Centigrade on gradual heating by the observer with the electromagnet signal. The mercury bulb of the thermometer was placed very close to the piece examined and both were placed in the beaker which was filled with 100 cc. aqueous media and was gradually heated in a water bath. The beaker used as the bath vessel was ca. 500 cc. capacity and contained 350 cc. water in which the former smaller beaker was immersed until the surface of the liquid in the former was a little lower than that of the latter.

According to W. KÜHNE (1864), the heat shortening will occur at different temperatures according as the tissues are rapidly subjected to the high temperature or gradually accustomed to it, and there is a reciprocal relation between the temperature and the time of its subjection, in that the greater the temperature is, the less time is needed to produce the heat shortening. Though it was all but impossible for such a simple device as this to raise the temperature at an accurately constant rate and the following degree of ability of regulation was not, of course, completely satisfactory to me, by adjusting the burner of the alcohol lamp, I was able, except in the case of *Branchiura*, to keep the elevation of the temperature at the rate of 55–65 seconds per degree C. between 30° and 40°C. *Branchiura* was much affected by heat in rhythmic contraction of the body and became very brittle when it was exposed to high temperature for so long a

duration, so that in this case only, the worm was subjected to heat,
elevating the temperature at the rate of 35–40 seconds per degree C.

   Loaded weight also affected the rate of shortening in tissues. But
it was impossible to apply constantly the same weight to all species
of worms, since the weight of a piece and its power of shortening
are different in different species of worms. I have chosen the proper
weight for every species, so as to exhibit the sudden contraction of
tissues on gradual heating, but applied invariablly the same weight to
every piece of tissue so far as they belonged to the same species of
worm.

   Solutions used as media, in which the living isolated tissues were
immersed, were a 3.3 per cent solution of pure sodium chloride and
natural sea water for the marine polychaetes, and distilled water for
the terrestrial oligochaetes. The osmotic pressure of 3.3 per cent
solution of sodium chloride corresponds nearly to the concentration of
the sea water off Asamushi, the salinity of which varies between 32.62
and 33.78 at 15°C. through a year (1926) except in April and May
(S. HATAI and S. KOKUBO 1928) when the melting snow on mountains
in this district causes temporarily a greater amount of river water to
pour into the sea. The polychaetes for which natural sea water was
used are as follows:

Species	Salinity at 15°C.	Date
Arenicola cristata ♂	33.2–33.8	Sept. 1929.
Arenicola cristata ♀	33.6–34.4	Sept. 1928.
Cirratulus dasylophius	33.1–34.0	Oct. 1928.
Polynoë sp.	32.2–33.5	Nov. 1928.

The other polychaetes employed were heated in a 3.3 per cent solution
of sodium chloride.

   With the object of simplifying and keeping constant the environ-
mental conditions of the experiment, I employed such simple media
as described above. The pure solution of sodium chloride and distilled
water, of course, are not desirable media, as is well known, for the
maintenance of tissues in the most lively state for a long time, but
for a such short time as it takes for temperature to rise from ca. 25°
to 50°C. at the rate noted above, the injurious effect of these media
should be negligible.

   For the precies determination of the onset point of heat shortening

in the curve traced on the drum of the kimograph, firstly, I determined the point of contact E on the traced curve with the line AB (see Figure 1) drawn parallel to the base line (in this case the line drawn by the temperature marking lever) CD.

Figure 1

Next, a line was drawn through the point G, at which the line AB and the arc KGM intersected each other, and another point H, which was marked on the line of temperature recorded, when the kymograph was stopped. The arc KGM was part of the circle drawn by the shortening-curve tracing lever at the place, K, where the kymograph was stopped. Lastly a line was drawn through the point E and parallel to the line GH, and we found that the onset temperature corresponded to the point of sudden change of direction on the traced curve, at the crossing point F between the newly drawn line and the line of temperature recorded.

EXPERIMENTAL RESULTS

(1) *Nereis mictodonta* MARENZELLER

The worms were collected from the muddy coast of the village of Ōchō, Ōsaki-Simojima Island in Seto-Uchi in late winter (Feburuary) 1929. The specimens were divided into eight equal divisions together with the internal organs contained and each division was designated

A, B, C, etc. respectively according to the order of passing from head to tail end. The worms employed were 7.2 to 10.5 cm. in body length.

TABLE 1.[1]

Nos. of Worms	A	B	C	D	E	F	G	H
9	44.5	44.8	45.5	46.6	47.4	47.2	46.6	46.9
7	44.6	44.7	46.6	46.6	46.2	46.8	46.2	46.0
15	44.9	45.1	44.7	46.4	46.4	47.2	46.6	46.2
8	45.3	46.1	47.7	47.4	47.3	47.3	47.5	46.8
14	45.5	46.1	46.7	46.9	46.6	46.8	47.2	47.0
13	45.5	46.2	46.2	46.7	46.4	47.1	47.3	47.2
3	45.9	45.7	46.8	47.0	47.8	48.0	47.8	47.2
6	46.0	45.6	46.4	46.8	46.6	46.7	46.8	46.7
11	46.0	46.1	47.1	47.2	47.0	46.7	46.7	46.6
2	45.2	46.8	45.5	46.4	46.2	46.6	46.8	46.4
12	45.4	45.3	46.6	46.5	46.6	46.5	46.8	46.6
10	45.7	45.4	47.1	46.9	46.9	47.3	47.5	47.4
5	46.3	47.2	47.3	46.1	47.6	47.5	47.4	47.6
4	46.4	46.2	46.4	46.7	47.0	46.9	46.6	47.0
1	46.4	46.4	46.3	46.4	46.4	46.3	47.0	47.2
Average	45.6	45.8	46.4	46.7	46.8	47.0	47.0	46.8

As Table 1 shows, the onset temperature of heat shortening in *Nereis mictodonta* increases anteroposteriorly and reaches the maximum at the posterior divisions F and G, but again decreases in the caudal end H. (See Chart 2.)

(2) *Polynoë vexillaria* (MOOR)

The worms were collected from the bottom of about fifteen fathoms off Yunoshima Islet, near Asamushi, in early summer (June) 1929. The worms employed were 3.3 to 4.0 cm. in body length. The specimens were divided into six equal divisions of both dorsal and ventral sides of the body wall. The heat-shortening gradient in *Polynoë vexillaria* is very similar to that of *Nereis mictodonta*. (See Chart 3.)

[1] Figures given in Tables 1-21 indicate the heat-shortening temperatures, in degrees Centigrade.

TABLE 2 a.

Dorssal Side.

Nos. of Worms	A	B	C	D	E	F
3	37.7	38.1	38.8	38.5	37.6	37.6
4	38.3	38.3	38.4	38.7	38.4	38.2
2	38.6	38.6	39.3	39.6	39.6	38.8
1	38.0	37.9	38.3	39.1	38.9	38.1
Average	38.2	38.2	38.8	39.0	38.6	38.2

TABLE 2 b.

Ventral Side.

Nos. of Worms	A	B	C	D	E	F
4	37.5	38.7	39.0	38.8	38.8	38.5
3	38.5	38.8	39.0	39.4	39.4	39.2
2	37.7	38.7	38.6	39.5	39.5	38.9
1	38.1	37.5	38.5	39.2	39.5	39.1
Average	38.0	38.4	38.8	39.2	39.4	38.9

TABLE 2 c.

Average of Dorsal and Ventral.

Side of Body	A	B	C	D	E	F
Dorsal	38.2	38.2	38.8	39.0	38.6	38.2
Ventral	38.0	38.4	38.8	39.2	39.4	38.9
Average	38.1	38.3	38.8	39.1	39.0	38.55

(3) *Polynoë* sp.

The worms were collected from the rocky shore of Tsuchiya, near Asamushi, in late autumn (November) 1928. These specimens were very small, only 1.3 to 1.9 cm. in body length, so I could divide into but four equal divisions, with internal organs included.

TABLE 3.

Nos. of Worms	A	B	C	D
3	42.8	44.0	44.0	45.5
2	43.1	43.5	45.4	45.4
16	43.1	44.2	44.8	45.5
11	43.5	43.8	44.5	45.4
1	43.9	44.3	44.4	44.4
10	44.1	45.0	45.9	46.3
14	44.2	45.0	45.0	45.0
4	44.3	44.6	44.6	44.6
6	43.3	42.8	44.0	44.7
8	43.8	44.8	45.4	45.2
13	44.0	43.8	44.2	45.1
7	44.0	43.8	44.3	45.2
12	44.3	44.1	44.4	45.0
5	44.7	44.2	44.6	44.9
9	45.7	45.6	46.5	46.2
Average	43.9	44.2	44.8	45.3

From the data given in Table 3, we find that most specimens showed a gradient which is a most simple gradation sloped down anteriorly. (See Chart 4.) As this gradient form was gained from only four divisions, the gradient form of this species might have resembled that of the former species, *Polynoë vexillaria*, if it had been possible to divide the worm into six or eight pieces and each piece had been tested separately. The fact that among fifteen tests three cases showed the onset temperature higher at division C than at A, while in two others that of division C was equal to that of division D, seems to indicate the possibility of a gradient form which is similar to that of *Polyoë vexillaria*.

(4) *Marphysa iwamusi* IZUKA

The specimens were collected from the muddy coast of Iwashi-Jima,

near the harbor of Onomichi, Hiroshima Ken, in early spring (March) 1929. The worms examined were all of mature form, clearly having their reproductive products, eggs or sperms, and were 28 to 35 cm. in body length. First, I tried to test the sixteen divisions of equal length of both the dorsal and ventral sides of the body, thus making altogether thirty-two divisions, but it required too long a time to complete the tests over all these pieces separately and it was very difficult to gain satisfactory, fair results of sudden shortening from so many pieces without any one failure. Moreover, in this case, as Chart 1 shows, it seemed to give the same form of curve whether we examined all sixteen divisions or eight divisions, which were equally divided from the whole body along its axis on both ventral and dorsal sides.
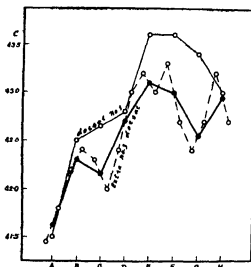
Chart 1. Heat-shortening Gradients of Marphysa iwamusi, showing nearly the same results obtained from two different observations, in the one of which the worm was divided into eight equal divisions (Dorsal No. 1) and in the other the worm was divided into sixteen equal divisions (Extra No. 3. Dorsal).

Therefore, I selected the latter method, dividing the worm into eight equal divisions as mentioned above, and the data given by such examinations were as follows:

TABLE 4 a. Dorsal Side.

Nos. of Worms	Sex	A	B	C	D	E	F	G	H
1	♂	41.5	42.5	42.6	42.8	42.6	43.6	43.4	43.0
2	♂	42.0	42.7	42.7	42.6	42.6	42.6	42.4	40.9
4	♂	42.7	43.0	43.1	43.4	43.6	43.2	43.1	42.9
7	♂	42.8	42.8	42.8	43.9	43.5	43.3	42.9	42.7
9	♀	41.2	42.0	42.1	42.1	43.0	42.5	43.2	42.3
6	♀	42.0	42.6	42.6	42.8	42.6	42.8	42.5	42.5
3	♀	42.5	42.7	43.1	42.9	42.2	42.9	42.2	42.2
8	♂	41.2	41.3	41.7	41.9	42.0	42.0	42.1	42.3
10	♀	41.6	42.0	42.8	43.0	42.6	43.5	42.2	42.6
5	♀	41.6	42.3	42.2	42.7	43.1	43.1	42.5	42.9
Average		41.9	42.4	42.6	42.8	42.9	43.0	42.7	42.4

TABLE 4 b. Ventral Side.

Nos. of Worms	Sex	A	B	C	D	E	F	G	H
6	♂	40.8	41.0	42.0	42.4	42.6	42.4	42.0	42.0
4	♂	40.9	41.3	41.7	42.0	42.0	42.3	42.0	41.9
9	♂	41.9	42.1	42.3	42.3	43.4	43.5	42.9	42.7
5	♂	41.5	41.5	42.9	43.7	42.8	43.1	43.3	42.3
1	♂	42.5	42.6	42.7	42.4	42.8	43.2	43.1	43.0
7	♀	41.5	41.3	41.8	42.0	43.0	42.7	41.7	42.1
3	♀	41.6	41.7	41.0	41.5	42.6	43.1	43.5	42.9
8	♀	42.2	41.6	42.5	42.5	42.9	43.2	43.2	43.2
10	♀	42.2	42.2	42.1	42.3	42.5	43.0	42.2	43.2
2	♀	43.1	42.6	42.5	42.7	42.9	43.0	42.8	42.9
Average		41.8	41.8	42.2	42.4	42.9	43.0	42.7	42.6

TABLE 4 c. Average Values.

Cases	A	B	C	D	E	F	G	H
Dorsal Average	41.9	42.4	42.6	42.8	42.9	43.0	42.7	42.4
Ventral Average	41.8	41.8	42.2	42.4	42.9	43.0	42.7	42.6
Male Average	41.8	42.1	42.4	42.4	43.0	42.9	42.7	42.5
Female Average	42.0	42.1	42.3	42.6	42.9	43.0	42.6	42.5
Total Average	41.85	42.1	42.4	42.6	42.9	43.0	42.7	42.5

As the above data show, there are no clear distinctions in the form of the heat-shortening gradient between male and female worms or between the dorsal and the ventral sides of the body. (See Charts 5 and 6.) In gradient form, based on the average values of all tests, the onset temperature is highest at division F, and decreases towards both extremities of the body.

I have also observed the heat-shortening gradient of the same species of worms collected from the rocky shore of Tsuchiya, near Asamushi, in late autumn (November) 1928. In this case I tested them in natural sea water, of which the salinity was 30.7 to 32.7 at 15°C.

TABLE 5 a.

Dorsal Side.

Nos of Worms	A	B	C	D	E	F	G	H
2	45.2	45.7	46.0	46.5	47.0	46.8	46.8	46.3
3	45.4	46.2	46.5	46.1	46.4	48.0	46.4	46.7
1	45.6	45.5	45.6	46.0	46.0	46.7	46.9	46.3
Average	45.4	45.8	46.0	46.2	46.5	47.2	46.7	46.4

TABLE 5 b.

Ventral Side.

Nos. of Worms	A	B	C	D	E	F	G	H
1	44.8	45.4	45.4	45.2	46.0	46.8	48.0	46.1
2	45.1	45.0	45.3	46.2	46.2	46.4	47.2	46.0
Average	45.0	45.2	45.4	45.7	46.1	46.6	47.6	46.1

TABLE 5 c.

Average of Dorsal and Ventral.

Side of Body	A	B	C	D	E	F	G	H
Dorsal	45.4	45 8	46.0	46.2	46.5	47.2	46.7	46.4
Ventral	45.0	45.2	45.4	45.7	46.1	46.6	47.6	46.1
Total Average	45.2	45 5	45.7	45.95	46.3	46.9	47.15	46.25

As the above tables show, the gradient form, based on the onset temperature of heat-shortening of the worms from Tsuchiya (Asamushi) resembles fairly closely that of the worms from Onomichi, though each part of the former specimens is higher in the onset temperature than the corresponding part of the latter. (See Chart 7.)

(5) *Nephthys caeca* MULLER

These specimens were collected from the sandy coast of a small fishing village, Moura, near Asamushi, in early summer (June) 1929. The worms used were 9.0 to 13.3 cm. in body length, and the worms were divided into eight equal divisions. As the contractile power of the body wall tissue is very poor though it is thick and relatively elastic, the rings of the body wall, without internal organs, were employed.

TABLE 6.

Nos. of Worms	A	B	C	D	E	F	G	H
9	37.5	38.0	38.4	37.2	37.2	37.5	38.0	39.1
2	38.0	39.2	40.1	39.1	38.3	37.5	38.1	38.4
6	38.2	40.2	39.8	39.4	37.6	37.3	37.7	38.3
5	38.3	38.9	38.9	39.1	38.4	38.5	39.4	39.5
8	38.4	39.3	39.1	38.7	38.6	38.3	39.0	39.6
10	38.2	39.2	39.8	38.6	38.4	38.8	38.3	38.9
7	38.3	39.5	39.7	39.0	37.6	37.7	37.8	37.3
1	38.9	38.9	40.4	39.4	39.0	39.7	40.2	40.0
4	38.9	38.7	39.0	37.9	37.7	37.2	37.6	37.2
3	38.9	38.7	39.0	38.3	37.9	37.2	37.5	37.1
Average	38.3	39.0	39.4	38.7	38.3	38.0	38.4	38.5

The gradient form of heat-shortening in this species is very similar to that of *Arenicola cristata* mentioned above. (See Chart 8.)

(6) *Glycera* sp.

The specimens were collected from the muddy coast in front of the Ōchō Fish-Cultural Station of Ōchō-Mura, Ōsaki-Simojima Island in late winter (Feburary) 1929. The worms were 9.5 to 15.0 cm. in body length. Each worm was divided into eight equal parts and was tested without internal organs as mentioned in the case of *Nephthys caeca*.

TABLE 7.

Nos of Worms	A	B	C	D	E	F	G	H
5	40.7	40.7	41.2	40.8	40.5	40.7	40.7	40.8
13	41.6	41.8	42.0	41.9	41.8	42.2	42.2	42.2
12	42.0	42.2	42.5	42.6	42.5	42.6	42.8	43.0
7	42.0	42.3	42.6	42.8	41.8	41.7	42.5	42.5
9	41.1	40.9	41.1	41.3	40.3	41.0	41.0	41.3
14	41.7	41.5	42.6	42.1	42.1	42.6	42.6	42.8
6	42.0	42.4	42.6	41.8	42.2	42.6	43.0	42.4
1	42.1	41.8	42.0	42.3	42.6	42.1	42.3	43.4
15	42.1	42.3	41.9	42.4	41.0	41.3	41.8	42.0
8	42.2	42.3	42.2	42.4	41.4	42.0	42.2	42.5
2	40.9	40.4	40.5	40.6	40.2	40.6	40.8	40.5
11	41.2	41.1	41.0	41.4	41.5	41.2	41.0	41.6
4	41.8	41.0	41.3	42.0	41.7	41.5	40.6	42.2
3	41.8	42.6	42.6	42.7	42.0	42.4	42.3	42.3
10	42.2	41.8	41.9	42.4	41.6	42.1	43.4	42.0
Average	41.7	41.7	41.9	42.0	41.6	41.8	41.9	42.1

The gradient form of *Glycera* sp. is similar to that of *Nephthys caeca* though the maximum height of the curve is a little lower than that of the latter. (See Chart 9.)

(7) *Cirratulus dasylophius* MARENZELLER

The worms were collected from the coast of Moura, near Asamushi, in mid-autumn (October) 1928. The specimens were divided into eight equal divisions together with internal organs. The body length ranged from 7.0 to 8.9 cm.

The gradient form of heat-shortening in this worm is in general similar to those of the above two species, with the exception of a

TABLE 8.

Nos. of Worms	A	B	C	D	E	F	G	H
15	46.4	47.2	47.4	48.2	49.0	46.2	47.9	48.1
4	47.0	48.7	49.0	47.4	46.8	47.0	49.1	49.2
10	47.8	48.6	48.5	48.5	48.3	47.0	48.8	49.0
14	46.3	48.0	47.0	48.3	47.9	48.4	48.4	48.8
2	47.0	46.9	47.7	42.8	44.3	44.5	48.6	48.7
11	47.4	47.6	47.1	46.9	45.4	48.8	47.0	48.3
3	47.5	48.9	47.1	47.0	45.2	48.3	48.3	48.7
6	47.6	48.7	48.8	48.5	48.6	48.2	49.2	49.3
7	47.8	46.7	47.6	46.7	47.4	47.4	49.0	48.8
12	47.8	48.2	47.9	47.8	46.0	48.3	48.2	49.2
13	48.3	47.5	47.3	45.6	45.8	48.2	48.6	48.6
8	46.9	47.9	47.8	47.2	49.3	49.2	48.9	48.6
1	47.4	46.6	47.2	45.2	47.7	44.6	48.8	49.4
5	47.6	48.5	47.8	48.1	46.8	47.1	48.4	48.3
9	47.8	47.3	48.0	46.8	47.9	47.1	48.9	48.7
Average	47.4	47.7	47.7	47.0	47.1	47.0	48.5	48.7

sudden greater rise of the curve at posterior region. (See Chart 10.)

(8) *Terebella* sp. (*T. debilis* ?)

These specimens were collected from the muddy bottom of about fifteen fathoms off Yunoshima Islet, near Asamushi, in late spring (May) and mid summer (July) 1929. The worms employed were 7.0 to 8.0 cm. in body length. The body of *Terebella* sp. is divisible into two parts with its external morphological features; the anterior half contains three segments of gill-bearing parts and, following, sixteen segments which have characteristic so-called "shields" thickend by the glandular tissues; the other half contains the posterior segments. The former was divided into four equal parts and the latter was divided into five equal parts.

I tested twenty spring worms and ten summer worms. The gradient form of *Terebella* sp. is also like those of the above three species. (See Chart 11.)

As the foregoing data show, *Terebella* sp. sets in heat shortening at a very low temperature (about. 29° to 33°C.). J. FRENZEL (1885) also detected such a low maximal temperature-limit of life (27° to 30°) in *Terebella*. Besides this species, *Myxicola infundibulum* also shows

a lower onset temperature than the other polychaetes. (See Table 14.).
I shall show them in the following figures.

Figure 2. Heat-Shortening Curve of *Terebella* sp. (Anterior Part)

Figure 3 Heat-Shortening Cruve of *Myxicola infundibulum* (Middle Part).

The onset temperature of heat-shortening of *Terebella* sp. is a little higher in summer worms than in spring worms as shown in Table 9 e.

TABLE 9 a.

Dorsal Side (in Spring Worms).

Nos. of Worms	A	B	C	D	E	F	G	H	I
6	29.5	30.9	31.0	30.9	30.8	30.6	30.5	31.0	31.1
3	30.8	31.0	31.9	31.8	31.8	31.3	30.0	30.6	31.5
7	31.2	32.1	32.7	32.4	32.1	41.6	31.0	31.7	32.4
2	29.8	30.5	31.8	30.5	32.2	29.4	28.5	28.8	29.0
10	30.2	30.2	30.1	30.5	31.0	30.0	30.7	30.7	31.4
5	31.0	30.6	31.4	31.2	32.4	32.3	30.9	31.3	31.4
4	32.1	31.6	32.0	31.8	31.7	31.2	30.5	30.7	31.3
1	28.4	30.5	26.1	31.4	32.1	28.7	29.1	28.3	28.5
8	30.5	32.2	30.0	32.0	32.5	31.8	31.1	31.6	31.0
9	32.0	30.0	30.5	31.3	30.9	31.8	31.6	30.1	31.5
Average	30.6	31.0	31.3	31.4	31.8	30.9	30.4	30.5	30.9

TABLE 9 b.

Ventral Side (in Spring Worms).

Nos. of Worms	A	B	C	D	E	F	G	H	I
8	28.8	30.5	32.6	32.1	31.9	30.3	31.3	32.0	32.0
4	30.0	30.8	30.9	31.5	32.2	31.4	30.7	30.7	31.6
7	30.6	30.6	30.9	30.9	31.6	31.1	30.6	30.9	31.3
1	27.0	28.1	29.2	29.1	29.5	29.0	28.8	29.2	29.5
2	29.0	30.1	31.1	31.4	31.0	28.7	29.1	29.5	29.2
5	29.5	31.1	31.8	30.7	32.1	30.6	28.6	29.1	31.3
10	32.5	33.0	32.2	32.9	31.9	31.2	31.7	32.6	33.2
7	29.9	31.3	30.5	31.6	32.0	31.7	31.8	31.4	31.5
9	30.0	30.6	31.4	30.5	32.4	31.2	31.3	31.0	31.5
8	30.1	29.4	29.2	30.2	30.9	29.3	28.8	29.5	30.5
Average	29.7	30.5	31.0	31.1	31.6	30.5	30.3	30.4	31.2

TABLE 9 c.

Dorsal Side (in Summer Worms).

Nos. of Worms	A	B	C	D	E	F	G	H	I
12	32.2	33.0	33.6	33.8	33.8	32.5	32.5	32.2	32.4
13	32.4	32.4	33.5	33.7	33.9	33.7	32.7	32.6	32.8
15	32.0	32.1	32.9	32.2	33.0	32.7	32.0	32.3	32.3
11	30.8	31.7	31.6	33.5	33.5	32.3	31.7	32.7	32.2
14	32.2	31.4	31.0	31.1	31.5	31.4	30.8	30.3	30.8
Average	31.9	32.0	32.5	32.9	33.1	32.5	31.9	32.1	32.1

TABLE 9 d.

Ventral Side (in Summer Worms).

Nos. of Worms	A	B	C	D	E	F	G	H	I
14	29.8	30.1	30.3	32.1	34.2	32.1	32.2	32.6	32.8
15	30.2	31.4	32.1	31.6	31.9	31.4	31.3	31.9	31.9
12	32.2	33.4	32.0	33.7	34.7	34.3	32.3	32.5	33.3
11	32.3	32.1	32.7	32.8	32.4	32.5	32.4	32.5	32.7
13	32.4	32.4	32.6	31.2	32.0	32.4	31.5	32.0	32.7
Average	31.4	31.9	31.9	32.5	33.0	32.5	31.9	32.3	32.7

TABLE 9 e.

Average Values.

Cases	A	B	C	D	E	F	G	H	I
Dorsal Average	31.0	31.3	31.7	31.9	32.2	31.4	30.9	31.0	31.3
Ventral Average	30.3	31.0	31.3	31.5	32.0	31.1	30.8	31.2	31.7
Spring Worms Average	30.15	30.75	31.15	31.25	31 7	30.7	30.25	30.45	31.05
Summer Worms Average	31.65	31 95	32.2	32.65	33.05	32.5	31.9	32.2	32.4
Average of Spring Worms and Summer Worms	30.9	31.35	31.68	32 0	32.38	31 6	31.13	31.33	31.73

(9) *Stylaroides plumosa* MÜLLER

The specimens were collected from the muddy bottom of about fifteen fathoms off Yunoshima Islet, near Asamushi, in early summer (June) 1929. The worms employed were 3.0 to 3.7 cm. in body length, and were divided into six equal divisions. The heat shortening gradient in *Stylaroides plumosa* resembles the sine-curve. (See Chart 12.)

TABLE 10 a.

Dorsal Side.

Nos. of Worms	A	B	C	D	E	F
9	38.0	39.4	38.2	38.2	38.1	38.7
7	38.2	38.6	40.5	39.7	38.5	40.5
13	38.5	39.3	38.7	38.6	38.0	38.9
3	39.7	40.8	40.3	39.3	38.9	39.5
1	39.7	41.4	41.0	39.7	39.2	40.4
18	40.0	40.2	40.2	40.0	38.4	39.8
6	38.1	40.3	39.0	39.4	38.8	39.7
5	39.0	38.9	39.4	39.2	39.2	39.9
11	39.2	39.5	38.0	38.2	37.9	39.0
12	39.5	39.8	38.3	38.5	38.0	39.1
14	39.9	40.4	39.8	39.9	38.9	39.4
2	40.4	41.2	39.8	40.2	39.0	39.3
8	40.5	40.3	41.2	38.0	38.2	38.8
10	37.9	38.7 ·	38.6	40.0	39.2	38.3
Average	**38.9**	**39.9**	**39.5**	**39.1**	**38.6**	**39.4**

TABLE 10 b.

Ventral Side.

Nos of Worms	A	B	C	D	E	F
1	36.6	38.6	39.6	39.4	38.1	38.3
5	38.5	39.1	39.4	39.2	38.6	38.9
12	38.6	39.2	38.7	38.5	39.0	39.3
2	38.8	39.2	39.2	39.0	37.8	38.1
15	39.1	39.5	40.0	39.0	38.3	38.7
3	39.4	39.6	39.5	39.2	39.4	39.5
4	39.4	40.0	40.6	39.8	38.9	39.8
7	39.5	39.5	40.4	39.4	38.4	38.9
13	39.5	40.4	40.0	38.9	38.2	38.9
10	39.8	40.4	39.4	39.2	39.6	39.6
11	40.0	40.0	40.3	38.5	37.8	38.6
14	39.0	39.7	38.4	37.5	38.0	37.9
6	39.7	40.1	38.7	39.4	38.4	38.6
9	40.0	39.9	40.2	39.5	38.5	39.9
8	40.3	40.7	37.7	38.0	39.6	40.0
Average	39.2	39.7	39.5	39.0	38.6	39.0

TABLE 10 c.

Average of Dorsal and Ventral.

Side of Body	A	B	C	D	E	F
Dorsal	38.9	39.9	39.5	39.1	38.6	39.4
Ventral	39.2	39.7	39.5	39.0	38.6	39.0
Average	39.05	39.8	39.5	39.05	38.6	39.2

(10) *Arenicola cristata* STIMPSON

The worms were collected from the sandy coast of Moura, a small fishing village, and that of Urashima, a small bay by the village of Nonai, near Asamushi, in early autumn (September) 1928 and 1929. The worms were 8.0 to 9.5 cm. in body length. The body of *Arenicola cristata* may be divisible into three different parts morphologically; the anterior part bearing parapodia but no gills, the middle part bearing both parapodia and gills, and the posterior part bearing neither para-

podia nor gills. The anterior part was divided into two divisions designated A and B; division A contained segments I-IV, division B, segments V-VII. The middle part was divided into three divisions C, D and E; C contained segments VIII-XI, D, segments XII-XIV, E, segments XV-XVIII. The posterior part was divided into two equal divisions F and G. The dorsal body wall alone was examined here.

TABLE 11 a.

Male Worms.

Nos. of Worms	A	B	C	D	E	F	G
2	43.1	44.6	44.7	45.9	44.5	42.8	43.2
1	43.6	44.4	44.7	45.4	44.5	41.3	43.1
9	43.6	44.2	44 3	45.7	45.8	42.8	43.7
8	43.6	45.6	45.1	45.8	43.8	43.5	43.9
10	44.0	45.1	45.8	46.0	45.0	43.1	44.2
5	44.2	44.5	45.0	45.7	44.2	42.0	44.9
3	44.2	44.7	44.9	45.3	44.7	42.8	43.1
4	44.5	44.6	45.1	45.0	44.6	43.9	44.3
6	44.7	44.9	46.0	45.9	45.2	43.2	44.5
7	44.1	45.1	45.3	45.6	46.0	44 6	43.6
Average	44.0	44.8	45.1	45.6	45.0	43.0	43.9

TABLE 11 b.

Female Worms.

Nos. of Worms	A	B	C	D	E	F	G
2	42.6	44.2	44.2	45.1	44.7	43 1	43.9
7	43.0	44.6	44.8	45.5	44.5	42.5	43.3
6	43.3	43.8	44.5	45.2	45.5	42.8	43.7
8	43.4	44.4	45.4	45.5	44.6	43.0	43.7
1	43.4	44.5	44.9	44.7	44.2	42.9	43.1
10	43.6	44.2	45.4	45.6	44.8	43.2	43.8
3	44.0	44.3	45.3	45.6	45.4	44.1	43.4
9	44.4	44.6	45.1	45.7	45.4	42.7	43.9
4	43.3	45.3	45.0	45.3	44.6	43.0	43.8
5	44.4	45.0	45.3	45.8	44.6	44.4	44.4
Average	43.5	44.5	45.0	45.4	44.8	43.2	43.5

TABLE 11 c.

Average of Male and Female.

Sex	A	B	C	D	E	F	G
Male	44 0	44.8	45 1	45.6	45.0	43.0	43.9
Female	43.5	44.5	45.0	45.4	44.8	43.2	43.5
Averag	43.75	44.65	45.05	45.5	44.9	43.1	43.7

The form of heat-shortening gradient in this species has already been described in the introduction of this paper. (See Chart 13.)

(11) *Praxillella* sp.

The worms were collected from the muddy coast of Iwashi-Jima Island, near the harbor of Onomichi in early spring (March) 1929. The worms were divided into six divisions; the first division, A, contained the anterior first four segments (I-IV); the second division, B, the following three segments (V-VII); the third division, C, three segments (VIII-X); the fourth division, D, three segments (XI-XIII); the fifth division, E, four segments (XIV-XVII), and the last division, F, four segments (XVIII-XXI). The worms employed were 7.0 to 9.0 cm. in body length. (See Chart 14.)

TABLE 12.

Nos. of Worms	A	B	C	D	E	F
8	38.7	39.3	39.5	39.6	39.3	39.3
3	39.2	39.4	40.4	40.3	39.9	40.4
4	39.2	40.0	40.2	40.2	40.2	39.9
1	39.2	40.4	40.4	39.7	39.7	39.8
2	39.3	39.7	39.8	39.3	39.3	39.5
6	39.4	39.5	39.8	40.1	39.0	39.0
10	39.4	39.8	40.2	40.4	40.1	40.0
5	39.6	39.6	40.1	40.2	40.0	40.0
7	39.7	40.5	40.3	40.4	40.3	40.0
9	39.8	40.2	40.0	40.1	40.2	40.0
Average	39.4	39.8	40.2	40.0	39.8	39.8

(12) *Potamilla myriops* MARENZELLER

The worms were collected from the muddy bottom of about fifteen fathoms off Yunoshima Islet together with common bivalves *Modiolus* in early summer (June) 1929. The worms were 3.2 to 5.5 cm. in body length. They were rather small considering that they are all of complete adult form.

TABLE 13 a.

Dorsal Side.

Nos of Worms	A	B	C	D	E	F	G	H
5	39.2	38.9	38.9	38.3	38.3	38.8	39.6	40.0
7	39.4	38.6	38.4	38.4	39.0	39.1	39.9	40.6
14	39.6	39.5	38.5	37.7	37.7	38.7	39.1	39.6
11	40.1	39.7	39.7	38.7	39.6	39.7	39.9	40.6
9	40.2	40.2	39.7	38.7	39.6	39.7	39.9	40.6
1	40.4	39.6	39.5	38.4	38.6	39.5	39.7	39.8
15	40.5	39.5	39.1	38.0	38.2	38.7	40.0	40.5
8	39.6	39.0	39.1	38.9	38.3	40.0	40.2	41.2
6	39.6	40.3	38.8	39.3	39.6	39.5	39.6	40.6
14	39.9	39.7	38.7	38.7	38.5	38.8	38.7	39.4
12	41.0	41.6	39.9	40.7	40.7	41.1	41.3	42.2
10	41.9	40.9	39.7	38.9	39.8	39.3	39.5	42.6
4	38.8	39.5	37.3	37.2	37.3	38.3	37.9	41.3
8	39.0	38.8	38.9	39.4	38.8	39.4	39.9	39.7
2	40.1	41.0	39.2	39.4	39.0	38.3	39.7	40.9
Average	40.0	39.8	39.5	38.8	38.9	39.3	39.7	40.7

TABLE 13 b.

Ventral Side.

Nos of Worms	A	B	C	D	E	F	G	H
6	39.8	39.6	38.4	37.2	38.1	38.5	39.9	40 0
7	40.2	39.5	39.4	39.3	38.8	39.0	39.5	39.5
14	40.4	40.2	39.5	39.4	38.9	39.2	39.5	39.9
5	39.4	38.3	38.0	38.7	38.3	39.2	39.7	40.1
3	39.5	39.7	39.6	39.7	38.9	39.0	39.6	40.2
10	40.5	39.3	39.6	39.6	39.4	39.7	40.3	41.5
2	40.5	39.0	38.5	39.8	38.3	39.1	39.3	40.0
1	40.5	39.6	39.0	39.0	41.1	39.2	40.1	40.4
11	40.7	40.6	39.0	38.9	39.8	40.5	41.0	40.5
15	40.8	39.5	39.3	39.0	39.2	39.6	39.2	41.3

8	41.5	40.0	39.7	39.7	40.5	40.6	40.4	41.0
12	41.7	40.4	41.4	40.9	41.2	41.2	41.3	42.0
9	41.8	40.7	39.4	39.3	39.6	40.0	42.4	42.2
13	38.2	39.6	39.4	39.0	39.0	39.2	38.9	39.3
4	39.4	40.2	38.6	38.3	38.3	38.2	39.4	39.1
Average	40.3	39.7	39.3	39.2	39.3	39.5	40.0	40.5

TABLE 13 c.

Average of Dorsal and Ventral.

Side of Body	A	B	C	D	E	F	G	H
Dorsal	40.0	39.8	39.5	38.8	38.9	39.3	39.7	40.7
Ventral	40.3	39.7	39.3	39.2	39.3	39.5	40.0	40.5
Average	40.15	39.75	39.4	39.0	39.1	39.4	39.85	40.6

As will seen from Chart 15, this species shows a simple bipolar gradient.

(13) *Myxicola infundibulum* MONTAGUE

The worms were collected from the muddy bottom of about fifteen fathoms off Yunoshima Islet in late spring (May) and early summer (July) 1929. The body length of the worms employed was 3.0 to 4.2 cm. The body of each worm was divided into six equal divisions together with internal organs.

TABLE 14.

Nos. of Worms	A	B	C	D	E	F
14	32.9	32.7	32.7	32.6	32.4	33.5
13	33.6	32.7	31.9	31.9	34.5	34.5
15	33.6	32.7	31.9	33.4	34.3	35.0
7	34.4	34.4	33.3	34.0	34.9	35.6
8	34.6	34.4	33.1	34.0	34.3	35.2
10	34.9	34.6	34.2	34.3	34.4	35.6
6	35.0	34.2	33.4	34.7	35.2	35.4
12	35.2	34.2	34.0	34.5	34.8	35.9
2	35.6	34.1	32.7	32.9	33.8	34.9

4	33.0	33.6	33.5	34.4	35.3	35.4
11	33.7	33.4	33.6	33.6	35.6	34.9
9	34.0	33.4	33.9	33.5	35.0	34.6
3	34.7	34.0	33.0	33.4	34.8	34.0
1	35.0	34.4	34.6	35.6	33.9	35.0
5	35.4	33.4	33.8	33.8	33.6	33.5
Average	34.4	33.8	33.3	33.8	34.5	34.8

The heat-shortening gradient in this species is also bipolar and V-shaped. (See Chart 16.)

(14) *Branchiura* sp.

The specimens were supplied by T. OKADA who was so kind as to collect them from the gutters about the outskirts of the city of Sendai in late autumn (November) 1928. The worms employed were 5.2 to 6.5 cm. in body length. The body of this worm was divided into eight equal divisions.

TABLE 15.

Nos. of Worms	A	B	C	D	E	F	G	H
2	45.4	44.7	44.6	44.7	44.0	43.9	43.6	43.3
9	45.5	44.3	44.3	43.8	43.7	43.6	42.6	42.9
1	45.8	44.7	45.2	44.3	44.5	44.2	44.2	43.8
7	46.0	45.5	45.1	45.0	44.5	43.6	43.0	43.4
15	46.2	44.6	44.3	44.0	43.2	42.4	43.3	43.0
8	46.6	45.7	45.2	44.6	43.9	43.7	43.9	43.1
13	46.8	44.6	44.6	43.7	43.2	42.6	43.1	42.6
4	44.0	44.4	43.3	43.0	43.2	42.6	44.0	43.0
6	44.9	43.9	43.0	43.9	43.0	43.1	43.1	43.0
14	45.5	45.1	44.0	43.3	44.4	43.8	42.9	42.2
10	45.8	44.8	44.4	44.8	44.0	44.1	43.6	43.5
11	46.4	44.7	44.8	43.7	43.6	43.3	42.7	43.5
12	46.5	45.8	45.7	43.8	43.9	43.6	43.8	43.9
3	45.4	43.5	43.6	43.1	44.0	42.7	43.5	43.0
5	46.0	45.0	44.6	43.3	43.6	43.2	43.6	43.6
Average	45.8	44.8	44.5	43.9	43.8	43.3	43.3	43.2

The heat-shortening gradient in *Branchiura* is a very simple monopolar type, showing the highest onset temperature at the head end and gradually decreasing towards the posterior anal end. (See Chart 17.)

Branchiura shows a higher onset temperature than the other ter-

retrial oligochaetes (HATAI, 1922), but the onset temperature of heat-
shortening in *Branchiura* detected by HATAI is a little higher (about
47°C on the average) than that (44.1°C. on the average) obtained
from the present experiments.

(15) *Pheretima communissima* (GOTO et HATAI)

The heat-shortening gradient, that of content of solid constituents,
and that of electrical potential in *Pheretima communissima* were tested
in 1927 at the physiological laboratory of the Medical Department of
the Hokkaidō Imperial University, Sapporo, by the courtesy of Prof.
H. MIYAZAKI and Prof. S. HOZAWA, Professors of Physiology in the
Medical Department there. The results obtained from the latter two
tests will be presented in succeeding pages. The worms were collected
from a field about the outskirts of the city of Sapporo, Hokkaidō, in
summer (July to August) 1927. The worms were 12 to 15 cm. in
body length. The body of the worm is divisible into two parts,
preclitellar and postclitellar; the former was divided into two equal
parts and the latter was divided into five equal parts and clitellar
segments were taken as one division, and designated *A*, *B*, *C*, etc.,
according to the order along the antero-posterior axis of the body,
as usual in other cases.

TABLE 16 a.

Dorsal Side.

Nos. of Worms	A	B	C	D	E	F	G	H
12	39.0		39.9		37.6		38.4	
17	39.4		37.1		36.1		38.0	
1	40.3		38.9		39.7		38.1	
16	40.3		39.1		39.7		38.8	
13	40.4		39.0		36.5		37.1	
19	40.5		39.4		39.5		39.2	
7	40.7		40.0		40.0		38.9	
9	40.9		41.0		40.5		38.7	
5	41.0		38.4		39.4		37.8	
3	41.1		37.7		37.5		39.5	
13		38.4		38.9		38.4		37.9
11		39.2		39.0		37.2		37.5
4		39.8		39.0		38.3		37.2
18		39.8		40.3		36.2		37.7
20		39.9		38.9		38.8		38.9
14		40.0		39.5		37.5		39.9

	A	B	C	D	E	F	G	H
10		40.1		39.3		38.6		38.0
2		40.2		40.5		39.0		37.7
6		40.5		40.7		40.1		39.2
8		40.7		40.2		40.3		40.6
Average	40.4	39.9	39.0	39.6	38.7	38.4	38.4	38.5

TABLE 16 b.

Ventral Side.

Nos. of Worms	A	B	C	D	E	F	G	H
8	38.7		38.2		37.6		35.7	
14	39.4		40.2		39.6		37.8	
2	39.5		37.2		38.8		37.8	
10	39.5		41.0		40.6		40.3	
20	39.8		38.7		37.8		37.9	
15	39.9			39.7		37.8		37.9
7	40.1		38.6		38.4		38.8	
5	40.6		38.3		39.3		38.8	
12	40.6		38.9		37.6		36.5	
18	40.9		38.5		37.9		38.8	
19		37.9		38.0		37.8		38.3
13		38.2		39.3		38.8		39.3
1		38.8		37.9		38.6		38.7
3		39.1		38.6		36.5		36.8
6		39.1		38.8		37.5		38.4
9		39.4		38.9		35.9		38.6
17		39.6		38.3		36.4		36.8
11		40.1		39.8		37.6		39.5
4		40.5		38.2		36.4		38.2
16		41.0	37.7		36.8		39.7	38.3
Average	39.9	39.4	38.7	38.8	38.4	37.5	38.4	38.3

TABLE 16 c.

Average of Dorsal and Ventral.

Side of Body	A	B	C	D	E	F	G	H
Dorsal	40.4	39.9	39.0	39.6	38.7	38.4	38.4	38.5
Ventral	39.9	39.4	38.7	38.8	38.4	37.5	38.4	38.3
Average	40.15	39.65	38.85	39.2	38.55	37.95	38.4	38.4

The gradient from of heat-shortening in *Pheretima communissima*

is of a V-shaped bipolar type, showing a little posterior rise as shown
in *Pheretima megascolidioides* and *Pheretima divergens* (HATAI, 1924),
though the onset temperature of each part in the writer's experiments
is a little lower than of those in HATAI's work. The onset temperature
of the clitellar region is lower than the both neighbouring divisions.
It may be due to the particular construction of clitellar tissues. (See
Chart 18.)

(16) *Allolobophola foetida* (SAVIGNY)

These specimens were collected from the Asamushi hot-spring town,
one and a half kilometers south of the Marine Biological Station, in
early winter (November) 1928. The worms employed were 6.0 to
8.2 cm. in body length. And the worms were divided into seven
divisions; the preclitellar part was divided into two equal divisions,
and clitellar part was cut out as one divisions, and postclitellar part
was divided into four equal divisions.

TABLE 17 a.

Dorsal Side.

Nos. of Worms	A	B	C	D	E	F	G
10	39.8	39.7	39.4	39.4	39.2	39.1	39.8
15	40.1	39.7	39.0	39.3	40.0	40.1	40.3
8	40.1	39.8	39.5	39.5	39.8	40.0	40.0
8	39.1	39.0	38.5	38.0	38.4	38.5	38.4
7	39.8	40.0	39.7	39.2	38.3	38.5	38.5
2	40.4	40.1	39.8	38.4	39.4	39.5	39.0
12	40.5	40.4	40.5	40.4	39.6	40.0	40.1
11	40.6	39.7	39.8	39.0	39.0	40.0	40.1
6	41.0	40.0	39.8	39.0	39.4	39.6	39.8
13	40.0	40.1	39.6	39.0	39.8	39.4	39.7
1	40.0	40.1	39.9	39.8	40.4	38.8	40.4
4	40.2	39.0	40.0	39.5	39.6	39.4	39.2
9	40.4	39.5	39.7	39.1	39.2	39.8	39.6
14	40.4	39.7	40.2	39.6	40.0	40.3	39.7
5	41.8	40.8	40.5	41.3	40.9	40.8	39.8
Average	40.3	39.8	39.7	39.4	39.5	39.6	39.8

TABLE 17 b.

Ventral Side.

Nos. of Worms	A	B	C	D	E	F	G
3	39.6	39.4	39.4	39.3	39.4	39.5	39.7
14	39.9	39.7	39.5	39.2	38.8	39.0	39.3
1	40.1	40.0	39.9	39.8	39.7	40.2	40.2
10	40.5	40.5	40.3	40.0	40.0	40.4	40.5
4	40.7	40.6	40.2	40.0	39.5	40.2	40.6
15	39.2	39.6	39.6	39.1	39.0	39.0	39.5
12	39.5	39.6	39.6	39.2	39.3	39.3	39.3
9	39.6	39.2	39.2	38.7	39.2	39.4	39.3
13	39.8	39.5	39.6	39.2	39.1	39.2	39.7
7	40.0	39.7	39.2	39.4	39.6	39.9	39.3
6	40.0	39.8	39.8	39.9	40.3	40.1	40.4
11	40.4	39.9	40.2	39.8	39.7	39.5	40.3
2	40.6	40.1	39.8	40.4	39.6	40.0	40.1
8	39.9	40.0	39.7	39.7	39.5	39.8	39.7
5	40.6	40.0	40.2	40.3	38.8	40.0	39.6
Average	40.0	39.8	39.7	39.5	39.8	39.7	39.8

TABLE 17 c.

Average of Dorsal and Ventral.

Side of Body	A	B	C	D	E	F	G
Dorsal	40.3	39.8	39.7	39.4	39.5	39.6	39.8
Ventral	40.0	39.8	39.7	39.5	39.4	39.7	39.8
Average	40.15	39.8	39.7	39.45	39.45	39.65	39.8

Allolobophora foetida also shows the V-shaped gradient in heat-shortening temperature. (See Chart 19.)

(17) Allolobophora caliginosa (SAVIGNY)

The specimens were collected from the town of Asamushi in early autumn (September) 1929. The body of the worm is divided into seven divisions in the same manner as in the case of Allolobophora foetida. The body length of the worms employed was 10 to 13 cm.
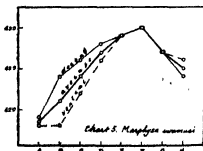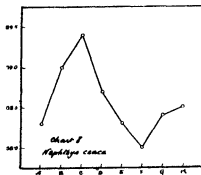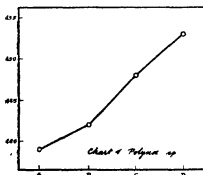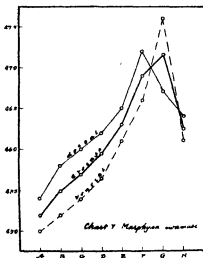
TABLE 18 a.

Dorsal Side.

Nos. of Worms	A	B	C	D	E	F	G
8	41.2	41.2	40.9	40.8	40.8	41.7	42.0
2	41.3	41.3	40.3	40.4	41.0	41.5	41.9
9	41.7	41.2	40.5	40.5	40.5	41.2	41.9
6	41.9	41.0	40.7	40.6	40.6	41.1	42.2
5	40.7	40.5	40.7	40.3	40.5	40.7	40.9
1	41.1	41.4	41.2	40.4	41.1	41.6	42.2
5	41.2	40.9	40.6	40.4	41.2	42.1	41.6
4	41.3	41.3	40.9	40.7	41.3	40.7	41.9
3	41.4	41.2	40.4	40.8	40.7	41.1	41.5
10	41.7	40.7	40.6	40.9	41.5	40.8	41.8
Average	41.4	41.1	40.7	40.6	40.9	41.3	41.8

TABLE 18 b.

Ventral Side.

Nos. of Worms	A	B	C	D	E	F	G
5	41.5	40.7	40.2	40.2	40.7	41.4	41.8
1	41.5	41.3	41.0	41.0	41.5	41.8	42.2
8	41.5	41.4	41.0	40.2	40.6	41.2	41.4
3	41.8	41.2	42.0	42.0	42.1	42.2	42.8
9	40.7	41.1	41.1	40.8	41.2	41.3	41.7
7	41.5	41.2	40.8	41.2	41.7	41.7	41.6
10	41.6	41.9	41.7	40.9	41.3	41.4	41.9
6	41.7	41.4	41.4	41.5	41.5	41.3	41.8
4	41.6	41.3	41.4	41.4	41.0	42.2	42.1
2	41.7	41.2	41.1	41.2	40.6	41.5	41.3
Average	41.5	41.3	41.1	41.0	41.3	41.6	41.9

TABLE 18 c.

Average of Dorsal and Ventral.

Side of Body	A	B	C	D	E	F	G
Dorsal	41.4	41.1	40.7	40.6	40.9	41.8	41.8
Ventral	41.5	41.3	41.1	41.0	41.3	41.6	41.9
Average	41.45	41.2	40.9	41.8	41.1	41.45	41.85

Chart 2 *Nereis virens* m.

Chart 3 *Polynoë ocellata*

Chart 4 *Polynoë* sp.

Chart 5 *Marphysa sanguinea*

Chart 6 *Marphysa iwanovi*

Chart 7 *Marphysa sanguinea*

Chart 8 *Naphthys caeca*

Chart 9 Bipora sp

Chart 10
Cerratilia dactylina

Chart 12
Hydriroides plumosa

Chart 13.
Aronista vistora

Chart 11 Jassballa sp

Chart 14 Praniabella sp

Chart 15.
Potamilla myriops

Chart 18.
Phascolosoma commune

Chart 16
Myxicola infundibulum

Chart 19
Allolobophora foetida

Chart 17.
Arenicola sp

Chart 20
Allolobophora caliginosa

The heat-shortening gradient of this worm is very similar to that of *Allolobophora foetida*, but the posterior rise is more extensive in the former than in the latter. (See Chart 20.)

H. M. VERNON (1899) reported that in *Lumbricus terestris* which is closely related to *Allolobophora* the onset temperature of heat shortening is highest at the anterior part (30.0°C.) and decreases posteriorly (at the middle part, 38.5°, and at the posterior part, 37.0° and 38.8°C.)

GENERAL CONSIDERATIONS.

As the preceeding data show, the onset heat-shortening temperature of different parts of the body or body wall in chaetopods varies in the form of a gradient along the axis of the body, and there are four typical distinctions among these heat-shortening gradients.

The first type of heat-shortening gradient is that which is known as a monopolar or primary gradient in chaetopods and is the most simple gradient, i. e., the onset temperature is highest at the anterior end and decreases posteriorly with quite simple gradation. The second type of heat-shortening gradient is that which has been called bipolar or secondary type of gradient in chaetopods, in which the onset temperature is high at the anterior part and decreases towards the middle part, but again increases posteriorly. The remaining third and fourth types are new ones detected by the writer in this investigation. In the third type (see Charts 1, 2, 3, 5, 6, 7) the onset temperature is highest at about the middle part, decreasing towards both extremities of the body, so that the form of this third type gradient resembles an upside-down image of that of the second type. The fourth type (see Charts 8, 9, 10, 11, 12, 13) is a little more complex than the others. It shows a form as if the second and third types were united, putting the anterior half of the second type over the posterior half of the third type. The fourth type, therefore, consists of three portions along the axis of the body: the first portion is represented by the anterior portion of the body where the onset temperature ascends from the head end, increasing towards the middle part and reaches the first maximum at the other end of this portion; the second portion or middle part of the body immediately follows the former and here the onset temperature decreases posteriorly and reaches the second minimum at the end of the posterior side of this

portion; the third portion, which is represented by the remainder of
the body, posteriorly, reaching the second maximum at the posterior
caudal end.

It is very interesting and of great importance for the present problem
to note how these four types of heat-shortening gradients are related
with the different morphological types or habitates of chaetopods

According to the classification of polychaetes adopted by W. B.
BENHAM in the Cambrige Natural History Vol. II (1922), Polychaetes
are divided into two branches, Phanerocephala and Cryptocephala,
and the former branch contains five sub-orders and the latter, two,
of these, the species here examined by the writer belong to the
following four sub-orders:

Phanerocephala,
    Sub-Order 1. Nereidiformia.
        Nereidae   *Nereis mictodonta* MARENZELLER
        Aphroditidae   *Polynoë vexillaria* (MOOR)
                           Polynoë sp.
        Eunicidae   *Marphysa iwamusi* IZUKA
        Nephthydidae   *Nephthys caeca* MÜLLER
        Glyceridae   *Glycera* sp.
    Sub-Order 3. Terebelliformia.
        Cirratulidae   *Cirratulus dasylophius* MARENZELLER
        Terebellidae   *Terebella* sp. (*T. debilis ?*)
    Sub-Order 5. Scoleciformia.
        Chlorhamidae   *Stylaroides Plumasa* MÜLLER
        Arenicolidae   *Arenicola cristata* STIMPSON
        Maldanidae   *Praxillella* sp.
Cryptocephala,
    Sub-Order 6. Sabelliformia.
        Sabellidae   *Potamilla myriops* MARENZELLER
        Eriographidae   *Myxicola infundibulum* MONTAGUE

The remaining three sub-orders, Sub-Order 2. Spioniformia, Sub-
Order 4. Capitelliformia and Sub-Order 7. Hermelliformia are yet
untouched.

F. E. BEDDARD classifies oligochaetes into two sub-orders, Microdrili
and Macrodrili (the Cambridge Natural History Vol. II. 1922). In
Microdrili I have examined only one species, *Branchiura* sp. and in

Macrodrili, three species, *Pheretima communissima*, *Allolobophora foe-
tida* and *Allolobophora caliginosa* were tested.

For the sake of convenience in the description below, I wish to
call the types of chaetopod gradients as the first, second, third and
fourth types as mentioned above, instead of the monopolar and bipolar
types or the primary and secondary types as used up to now.

(1) Nereidiformia.

Nereidiformia contains three different types of gradients in heat
shortening, namely the second, third and fourth types. In the second
type of heat-shortening gradient is represented by *Nereis ezoensis*
IZUKA and *Ceratocephala osawai* IZUKA, which had been observed by
HATAI (1924).

The similarity of their gradient form in heat shortening is shown
in the following Chart.
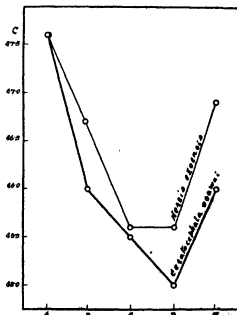
Chart 21. These graphs   based on the average values of
HATAI's observations (HATAI, 1924, p. 12).

S. NOMURA (1926) stated that in the four divisions of the body
of *Nereis japonica* IZUKA the percentage of solid substances was
greatest at the head division and decreased gradually posteriorly while

that of the content of total nitrogen, tested by the micro-KJEHDAHL method, decreased towards the middle of the body and reached the minimum, but again increased in the posterior division. In *Nereis virens* SARS and *Nereis vexillosa* GRUBE, the second type of gradient was shown in the rate of oxygen consumption by HYMAN and GALIGHER (1921) and in the electrical polarity by HYMAN and BELLAMY (1922). Summarizing these results of experiments the worms of the family Nereidae (Lycoridae) seem to belong to the second type in axial gradient, while the case of *Nereis mictodonta* shows the third type gradient in heat shortening as the only exceptional case in the family Nereidae thus far examined. (See Chart 2 & 22.)

The third type of gradient in heat shortening is also found in Nereidiformia such as *Polynoë vexillaria* of the family Aphroditidae, and *Marphysa iwamusi* of the family Eunicidae. The following chart shows the similarity of their gradient forms.
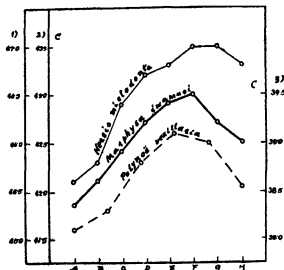
Chart 22. Scale 1) for *Nereis mictodonta*, scale 2) for *Marphysa iwamusi* and scal 3) for *Polynoë vexillaria*. These graphs are based on the data given in Tables 1, 2 c, and 4 c.

But the heat-shortening gradient of *Polynoë* sp. may be recognized as the intermediate type of gradient between the second and third types. (See Chart 3.)

Nephtys caeca and *Glycera* sp. are the representatives of the fourth type of gradient in Nereidiformia. See the following chart.
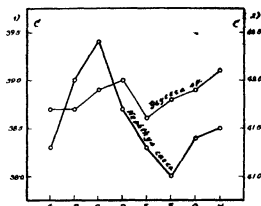
Chart 23   Scale 1) for *Nephthys caeca* and scale 2) for *Glycera* sp.
These graphs are based on the data given in Tables 6 and 7

(2) Terebelliformia.

Cirratulus dasylophius and *Terebella* sp. were examined here, and both show the fourth type of gradient in heat shortening. We see
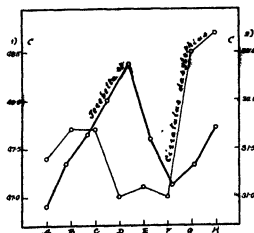
Chart 24. Scale 1) for *Cirratulus dasylophius* and scale 2) for *Terebella* sp. These graphs are based on the data given in Tables 8 and 9 e.

their similarity in gradient form in Chart 24.

(3) Scoleciformia.

In this sub-oder *Stylaroides plumosa*, *Arenicola cristata* and *Praxillella* sp. were examined. *Stylaroides plumosa* and *Arenicola cristata* show clearly the fourth type of gradient in heat shortening as already described in the introduction of this paper, while *Praxillella* sp. seems to belong to the second type when based on the average values alone. See the following chart.
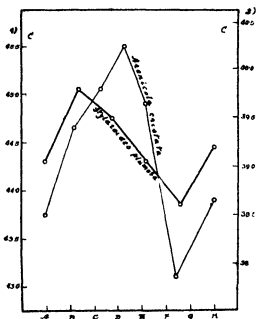
Chart 25. Scale 1) for *Arenicola cristata* and scale 2) for *Stylaroides plumosa*. These graphs are based on the data given in Tables 10 c and 11 c.

(4) Sabelliformia.

In this group I examined two species, *Potamilla myriops* and *Myxicola infundibulum*. These two species show the second type of gradient in heat shortening. According to HATAI (1924), *Laonome japonicus* which belong to this sub-order shows the first type (mono-polar) in heat-shortening gradient when the data obtained from the average values of the experiments on all five worms are adopted, but

one of them (No. 4, in the table on page 14, HATAI, 1924) crealy
showed the second type.
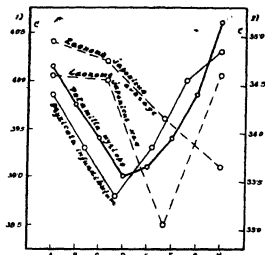
Chart 26. Scale 1) for *Laonome japonicus* and *Potamilla myriops*
and scale 2) for *Myxicola infundibulum.* The graphs of *Laonome*
japonicus are based on the data given by HATAI (1924, p. 14), and the
other graphs are based on the foregoing Tables 13 c, 14.

(5) Oligochaetes.

In oligochaetes, the heat-shortening gradient of *Branchiura* sp. is
of the first type and those of the other three species of macrodrilous
oligochaetes are of the second type. The facts that the gradients of
onligochaetes are of the first or second type are already demonstrated
on various lines of evidence by many authors up to date.[1] Later,
I shall comment a little upon the results of the different lines of
evidence.

However, in the case of *Branchiura* the gradient of the rate of
oxygen consumption and of CO_2 production observed on three parts

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[1] On Heat-Shortening Temperature and Content of Water by HATAI, S. (1924);
    On Susceptibility to Cyanides and Regenerative Power by HYMAN, L. H. (1916);
    On Respiration Rate by HYMAN, L. H. and A. E. GALIGHER (1921), SHEARER, C.
    (1924), OKADA, T. (1929); On Electrical Potential by MORGAN, T. H. and A. C.
    DIMON (1904), HYMAN, L. H. and A. W. BELLAMY (1922), WATANABE, Y. (1928);
    &c.

of the body by T. OKADA (1929) is not in accordance with that of
the heat-shortening temperature. My own result of heat shortening
shows the first type, while OKADA's result shows the second type or
a great posterior rise, similar to that shown by the susceptibility
gradients in other tubificids, *Tubifex rivulorum* LAMARCK and *Lim-
nodrilus claparedianus* RATZEL (HYMAN, 1916).

This discrepancy may be traced to the fact that, on comparing
the respiration rate of tissues, it seems to be necessary to take into
consideration the surface area as well as the amount of tissue. Since
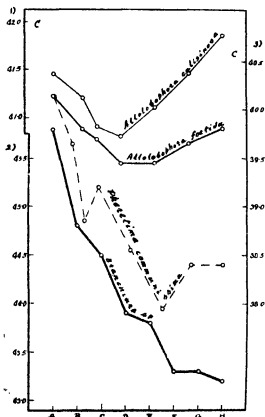
Chart 27. Scale 1) for *Allolobophora caliginosa*, scale 2) for *Bran-
chiura sp.*, and scale 3) for *Allolobophora foetida* and *Pheretima com-
munissima*. These graphs are based on the data given in Tables 15,
16 c, 17 c, and 18 c.

Branchiura has so-called gills of long slender process at the posterior
part of the body, the surface area per unit weight may be extensively
larger in the pesterior part than in the anterior part and the middle
part. However, OKADA's experiments were not observed with the
respect to the surface area of the *Branchiura* body, and therefore, it
may be impossible from the data given by him to compare the
respiration rate (or intensity) of three parts of the body with each
other in respect to the axial gradient of the body. Consequently,
whether the gradient of heat shortening does or does not agree in
form with the gradient of the respiration rate in *Branchiura*, unless
the amount of oxygen consumed or that of CO_2 produced per unit
area is determined, can not be definitely decided.

As already mentioned above, polychaetes were heated in natural
sea water, or 3.3 per cent solution of sodium chloride, while oligo-
chaetes were heated in distilled water. In case of comparing the
results of experiments done in such different media, it may be neces-
sary to examine into whether such comparisons, under the circumstance
mentioned above, be possible or not. Regarding this point, the follow-
ing simple examinations were undertaken.

I have examined the onset temperature of heat shortening with
Cirratulus dasylophius in four different media, namely in natural sea
water, and 1.1, 2.2, and 3.3 per cent solutions of sodium chloride.
The following results were obtained:

TABLE   19 a.

Onset Temperature of Heat Shortening in *Cirratulus dasylophius*
in Natural Sea Water, Salinity of which
is 34.4 to 35.7.

Nos. of Worms	A	B	C	D	E	F	G	H
2	46.9	47.5	47.5	47.7	48.0	48.0	48.3	48.4
4	47.3	47.2	47.4	47.3	47.3	48.0	48.5	48.8
5	47.3	47.8	48.4	47.3	47.2	48.4	48.6	48.6
3	47.5	47.8	48.1	47.2	47.5	48.6	48.6	48.7
1	48.1	48.3	48.5	47.8	47.8	48.3	48.5	48.7
Average	47.5	47.7	48.0	47.5	47.6	48.3	48.5	48.5

TABLE 19 b.

Onset Temperature of Heat Shortening in *Cirratulus*
dasylophius in 3.3% Solution of NaCl.

Nos. of Worms	A	B	C	D	E	F	G	H
3	46.8	47.6	48.0	47.4	47.4	48.2	47.8	49.0
2	47.1	47.7	47.4	47.5	47.4	47.6	48.4	48.6
4	47.1	47.8	47.4	47.7	47.6	48.3	48.5	48.9
1	47.3	47.7	48.0	47.9	47.2	48.2	48.8	48.6
5	47.8	47.6	47.7	47.2	47.3	48.5	48.2	48.3
Average	47.2	47.7	47.7	47.5	47.4	48.2	48.3	48.7

TABLE 19 c.

Onset Temperature of Heat Shortening in *Cirratulus*
dasylophius in 2.2% Solution of NaCl.

Nos. of Worms	A	B	C	D	E	F	G	H
1	45.8	47.2	46.8	46.4	46.1	47.0	47.6	47.8
5	46.3	47.2	46.4	46.0	46.3	47.2	47.4	46.9
3	46.5	47.1	46.7	46.9	46.4	46.9	47.5	47.9
4	46.7	46.7	47.4	46.6	46.6	46.4	47.0	47.7
2	47.4	47.0	46.6	46.4	46.5	47.1	46.7	46.8
Average	46.5	47.0	46.8	46.5	46.4	46.9	47.3	47.4

TABLE 19 d.

Onset Temperature of Heat Shortening in *Cirratulus*
dasylophius in 1.1% Solution of NaCl.

Nos. of Worms	A	B	C	D	E	F	G	H
3	44.8	45.8	46.0	45.7	45.9	45.9	46.5	46.3
4	44.9	45.4	45.3	45.3	45.4	45.6	45.9	46.4
1	45.5	45.7	45.7	45.4	45.3	45.4	45.8	45.8
2	46.0	46.2	45.8	45.9	46.0	45.5	45.7	46.6
5	46.2	46.1	46.0	45.5	45.2	45.5	45.5	45.9
Average	45.5	45.8	45.8	45.5	45.6	45.6	45.9	46.2

Y. WATANABE
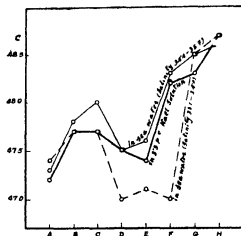
    Chart 28. Heat-shortening gradients of *Cirratulus daysilophius*,
placed in natural sea water and 3.3% NaCl Solution
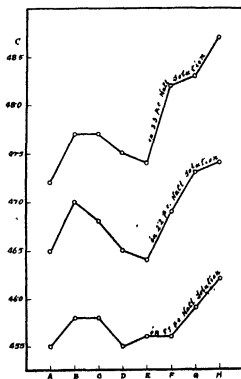
    Chart 29. Heat-shortening gradients of *Cirratulus daysilophius*,
placed in 1.1, 2.2, and 3.3, per cent Solutions of NaCl.

As the above data show, the heat-shortening temperature of each part of the *Cirratulus* body increases proportionally to the concentration of the solution of sodium chloride used as its media.

Such results were obtained also in the cases of terrestrial oligochaetes, *Allolobophora foetida* and *Allolobophora caliginosa*. The data in Tables 20 a, b, c show the results obtained from the experiments in 0.115 M solution of sodium chloride and those in Tables 20 b and 18 a, b, c show the results obtained from the experiments in distilled water.

0.115 M solution of sodium chloride, of which the degree of freezing point depression $\triangle$ is about 0.4, may be taken as isotonic to the body fluid of terrestrial annelids from the observation by L. FREDELICQ (1906) on the body fluid of *Hirudo officinalis*.

TABLE 20 a.

Onset Temperature of Heat Shortening in *Allolobophora foetida* in 0.115 M Solution of NaCl.

Nos of Worms	A	B	C	D	E	F	G
2	40.4	40.0	39.8	39.9	40.1	40.2	40.2
4	40.7	40.4	40.3	40.3	40.4	40.3	40.5
1	40.7	40.4	40.4	40.3	40.4	40.3	40.2
5	41.0	40.9	40.6	40.5	40.5	40.6	40.5
3	41.4	41.2	40.9	40.7	40.8	41.0	41.2
Average	40.8	40.6	40.4	40.3	40.4	40.5	40.5

TABLE 20 b.

Onset Temperature of Heat Shortening in *Allolobophora foetida* in Distilled Water.

Nos. of Worms	A	B	C	D	E	F	G
2	39.9	39.8	39.7	39.5	39.7	39.5	39.8
5	40.0	39.9	39.6	39.7	39.5	39.8	40.0
1	40.1	39.8	39.6	39.7	39.4	39.7	40.0
4	40.3	40.1	39.4	39.6	39.6	39.9	40.0
3	40.6	40.1	39.8	39.7	39.9	40.2	40.4
Average	40.2	39.9	39.6	39.6	39.6	39.8	40.0

TABLE 21 a.

Onset Temperature of Heat Shortening on the Dorsal
Side of *Allolophora caliginosa* in 0.115 M
Solution of NaCl.

Nos. of Worms	A	B	C	D	E	F	G
1	41.5	41.4	41.0	40.8	40.9	41.1	41.6
4	41.5	41.5	41.3	41.3	41.8	41.5	42.0
5	41.6	41.3	41.3	41.3	40.9	41.8	41.9
2	41.7	41.4	41.2	41.3	41.2	41.9	42.1
3	41.8	41.3	41.2	41.0	41.3	41.9	42.3
Average	41.6	41.4	41.2	41.3	41.2	41.5	42.0

TABLE 21 b.

Onset Temperature of Heat Shortening on the Ventral
Side of *Allolophora caliginosa* in 0.115 M
Solution of NaCl.

Nos. of Worms	A	B	C	D	E	F	G
5	41.5	41.3	41.2	41.2	41.1	41.1	41.2
4	41.6	41.4	41.3	41.1	41.4	41.6	42.6
3	41.9	41.4	41.3	41.4	40.9	41.5	41.7
2	41.9	42.0	41.4	41.4	41.5	42.0	42.9
1	41.2	42.1	41.3	41.4	41.7	42.5	42.5
Average	41.8	41.8	41.3	41.3	41.4	41.8	42.2

TABLE 21 c.

Average Values of Dorsal and Ventral.

Side of Body	A	B	C	D	E	F	G
Dorsal	41.6	41.4	41.2	41.3	41.2	41.5	42.0
Ventral	41.8	41.3	41.3	41.3	41.4	41.8	42.2
Average	41.7	41.35	41.25	41.3	41.3	41.65	42.1

As the experiment of VERNON (1899), of MOORE (1902), and of MEIGS (1909) have shown, the onset temperature of the heat-shortening may be altered by adding different amounts, as well as different kinds of salts. However, except with extremely improper media, any altera-
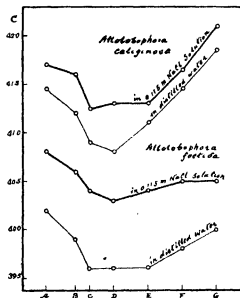
Chart 30. Heat-shortening gradients of *Allolobophora caliginosa* and *Allolobophora foetida*, placed in distilled water and 0.115 m solution of NaCl.

tions due to the difference of media used affect almost equally all the parts of the body and thus the relative values of the gradient should not alter to such extent as to materially modify the form of the curve. In reality, the heat-shortening gradient as a whole slightly altered its position on the temperature scale with different media, but the tendency of the specific form in the gradient of the heat-shortening temperature along the axis of the body for each worm was not altered. Therefore, the gradient form which I have determined may be taken as specific and characteristic for each species of worm.

The distribution of these four types of gradient forms in various groups of chaetopod annelids is conveniently summarized in the following table.

TABLE 22.

Distribution of Four Different Types of Axial Gradients in Chaetopod Annelids.

Sub-Orders	1st Type	2nd Type	3rd Type	4th Type
Nereidiformia	*Nereis japonica* 29) *Nereis limbata* (Larva) 5)	*Nereis essoensis* 15) *Nereis japonica* 29) *Nereis vexillosa* 18), 19) *Nereis virens* 18), 19) *Ceratocephala osawai* 15)	*Nereis nuciodonta* * *Marphysa iwanusi* * *Polynoë vexillaria* *	*Nephthys caeca* * *Glycera* sp *
		Polynoë sp *		
Spioniformia	*Chaetopterus pergamentaceus* (Larva) 6)	*Chaetopterus pergamentaceus* (Larva) 6)		
Terebelliformia				*Cirratulus dasylophus* * *Terebella debulis (?)**
Scoleciformia		*Arenicola cristata* (Larva) 6)		*Arenicola crustata* * *Stylaroides plumosa* *
			Praxillella sp *	
Sabelliformia	*Laonome japonicus* 15)	*Potamilla myriops* * *Myxicola infundibulum* *		
Microdrili	*Aeolosoma hemperichii* 17)	*Delo limosa* 17) *Delo frucata* 17) *Stylaria lacustris* 17) *Chaetogaster diaphanus* 17) *Nais elinguis* 17)		

		Lumbriculus inconstance 17)		
		Tubifex tubifex 17)		
		Tubifex rivulorum 17)		
	Branchiura sp. *	*Branchiura* sp. 30)		
		Pheretima communissima 36), *		
		Pheretima divergens 15)		
		Pheretima megascolidioides 15)		
Macrodrili		*Allolobophora caliginosa* *		
		(= *Helodrilus caliginosus* 18)		
		Allolobophora foetida 28), *		
		Lumbricus terrestris 28), 35)		

Note: Nos. noted under the specific name refer to the literature cited and asterisk indicates the species tested in this work.

After seeing the results of these experiments, we are confronted with the problems of what these four types of gradients signify and of whether any causal relations exist among them.

According to CHILD's experiment (1917) on the differential susceptibility in larvae of *Nereis limbata* and *Chaetopterus pergamentaceus*, at the beginning of developmenf the apical is most susceptible and the basal, least. As the development goes on, the posterior somatic plates show a relatively rapid increase in susceptibility, and as posterior elongation occurs, or soon after, the posterior growing region becomes the most susceptible region of the larval body. L. H. HYMAN (1916) showed that the susceptibility gradient of *Aeolosoma* is of the first type (monopolar), while those of naids, *Lumbriculus* and tubificids belong to the second type (bipolar). She also showed that the posterior rise is more extensive in *Lumbriculus* than in naids, and is most extensive in tubificids. And she found that these trans-

formations in the second type of chaetopod gradient also take place
during the course of growth in *Tubifex tubifex* LAMARCK. The sus-
ceptibility gradient in young *Tubifex* is of the second type, and the
most susceptible region of the body is the anterior end, while, as the
growth goes on, the posterior end becomes the most susceptible region
instead of the anterior region. From these facts shown by CHILD
and HYMAN, we see that in all probability the primodial gradient of
susceptibility in chaetopods is the first type of gradient and is modified
secondarily into the second type; then this posterior rise becomes
more and more extensive as the growth or the development proceeds.
And according also to them, such modifications of gradient type in
chaetopods are due to the result of the characteristic annelid method
of growth and development.

In the present work on the heat-shortening experiment, three
species of macrodrilous oligochaetes make a similar series with respect
to the axial gradient as shown by HYMAN on the susceptibility gradient
of microdrili. Genus *Pheretima* is considered to be more primitive in
the zoological scale than genus *Allolobophora* (see F. E. BEDDARD's
a Monograph of the Order of Oligochaeta, pp. 162–173 & fig. 34),
and the heat-shortening gradient of the latter shows a more extensive
posterior rise than that of the former, as shown in Chart 27.

However, we have found several cases in which the forms of the
gradient do not follow the zoological scale so far as the heat shortening
is concerned.

For instance, *Laonome japonicus* and *Potamilla myriops* are very
closely related worms, belonging to the same family Sabellidae, but
the heat-shortening gradient of the former is of the first type and
that of the latter is of the second type. (See Chart 26.)

Nereis mictodonta, as noted above, show the third type of gradient
in heat-shortening temperature while other species of the genus *Nereis*
and the related worm, *Ceratocephala*, show the second type of gradient.
(See Chart 21.)

The heat-shortening gradient of *Polynoë vexillaria* is of the third
type, while *Polynoë* sp. shows a simple linear gradation, sloped down
anteriorly; accordingly we can not determine whether the latter
belongs to the second type or the third type of gradient.

CHILD (1917) showed that the susceptibility gradient in the larva

of *Arenicola cristata* is of the second type, while the heat-shortening gradient in its adult form is clearly of the fourth type.

The heat-shortening gradient of *Praxillella* sp., which belongs to the same sub-order of Scoleciformia as *Arenicola* does, may be taken rather as an intermediate or indeterminable form, between the third and fourth type gradients, than as a perfect example of the third type gradient. On looking over every datum in Table 12, shown by this species, we find that at the two divisions of the most posterior part, *E*, *F*, among ten worms tested three showed a higher onset temperature at the posterior end, *F*, than at the other, *E*. Three other worms showed equal onset tempature at both divisions, while the remaining four alone showed a lower onset temperature at the posterior end, as is shown in the typical form of the third type of chaetopod gradient.

Thus the presence of several forms of gradients in the same group of related worms and of the same gradient forms in worms belonging to systematically different groups, seems to suggest that the modifications of the gradient form are not strictly associated with the phylogenetic relation of worms, so far as the observations on the adult worms alone were concerned. For the decisive resolution of these lines of question, observations on the transformations of form of the heat-shortening gradient which appear during the whole course of individual development or growth are necessary. Until the data on the embryos and larvae are obtained, with a suitable technique, the writer wishes to reserve any definite conclusion on this fundamental problem.

I remark here, in addition, that, in eight species examined, the form of the heat-shortening gradient did not differ in the different sides of the body (dorsal and ventral) in the same species of worm. (See Charts 3, 5, 7, 11, 12, 15, 18, 19, 20.) In three species, *Polynoё vexillaria*, *Potamilla myriops* and *Allolobophara caliginosa*, the ventral body-wall tissues are higher in the onset temperature of heat shortening than the corresponding tissues of the dorsal side; on the contrary, *Marphysa iwamusi*, *Terebellides* sp. and *Pheretima communissima*, the heat-shortening temperature of the dorsal body-wall tissues are higher than those of the corresponding tissues of the ventral side of the body wall; while in *Stylaroides plumosa* and *Allolobophora foetida*,

the heat-shortening temperatures are almost the same in the corresponding tissues of both the dorsal and the ventral side of the body wall. Accordingly, in the present research any regular relation concerning the difference of the heat-shortening temperature in the corresponding tissues of the dorsal and the ventral sides of the body wall, was not found.

According to CHILD, it is not necessary to assume that the gradients consist primarily in metabolic difference alone. He said in his book (1924), "Protoplasm is a system in which the chemical reactions of metabolism are so intimately associated with other factors, e. g., colloidal dispersion, active mass of enzyme, permeability of limiting surface, electrolytic content and dissociation, water content, etc. that to distinguish one particular factor rather than another as primarily is at present impossible." (p. 74.)

Some instances of such correlations as were suggested by CHILD are cited here from the writer's observations on the content of solid substances and the electrical potentiality in the earthworm, *Pheretima communissima*.

1) The work of HATAI (1924) in the earthworms, *Pheretima megas-*

TABLE 23.

Species			P. megascolidoides		P. communissima	
Experiments			Heat shortening	Content of solid substance	Heat shortening	Content of solid substance
Units			Degree C	%	Degree C	%
Ant.	I	A	41.2	21.1	40.2	21.2
	II	B	41.2	20.9	39.7	20.3
Cht.	III	C	40.5	25.2	38.9	23.4
Mid.	IV		39.7	19.6		
	V	D	39.4	18.3	39.2	19.5
	VI	E	39.1	18.4	38.6	18.8
	VII		39.0	17.7		
	VIII	F	39.0	17.7	38.0	18.3
	IX		38.9	18.1		
		G			38.4	17.3
Post.	X		38.9	18.3		
	XI	H	39.4	19.0	38.4	18.0

colidioides (GOTO et HATAI), and *Pheretima divergens* (MICHAELISEN)
showed that, "the temperature of the onset heat contraction is pro-
portional to the amount of solid constituents which are contained in
tissues." (p. 19.) In the other species of the genus *Pheretima*, P.
communissima, I have certified the same relation between the onset
temperature of heat shortening and the content of solid substance in
the tissues. The data given in Table 23 show this intimate correlation.

In the above table, data in the case of P. *megascolidioides* were
adopted from the work of HATAI (1924) p. 8. (the average of the
value of the dorsal and the ventral sides of the body of P. *megas-
colidioides* No. 4.) and from the first row of table on page 15; data
in the third column, from the foregoing Table 17, and those in the
fourth column, from the following Table 24.

TABLE 24 a.

Contents of Solid Constituents in Different Parts of
the Body in *Pheretima communissima*, given
as Percentage of the Fresh Tissue.

Dorsal Side.

Nos. of Exper	A	B	C	D	E	F	G	H
3	20.2	19.7	23.1	19.0	19.3	19.1	17.4	16.6
4	20.3	20.1	21.7	17.5	17.6	16.6	17.0	16.8
5	21.4	20.6	23.8	20.0	19.1	18.6	17.5	17.8
2	21.9	20.6	23.7	20.8	19.4	18.4	17.7	18.2
1	22.1	21.0	25.0	19.7	18.5	18.2	17.4	18.7
Average	21.2	20.4	23.5	19.3	18.8	18.2	17.4	17.7

TABLE 24 b.

Ventral Side.

Nos. of Exper.	A	B	C	D	E	F	G	H
4	20.4	20.1	20.2	17.9	17.6	16.9	17.3	18.3
3	20.4	20.8	24.2	20.2	19.3	18.8	17.6	18.2
5	21.5	20.8	24.0	20.1	19.0	18.7	17.2	18.3
1	21.7	19.2	24.4	19.2	18.6	19.0	16.5	18.0
2	22.3	20.9	26.3	20.7	18.9	18.1	17.4	18.7
Average	21.1	20.1	23.3	19.6	18.7	18.3	17.7	18.3

TABLE 24 c.

Average of Dorsal and Ventral.

Side of Body	A	B	C	D	E	F	G	H
Dorsal	21.2	20.4	23.5	19.3	18.8	18.2	17.4	17.7
Ventral	21.1	20.1	23.3	19.6	18.7	18.3	17.1	18.3
Average	21.15	20.25	23.4	19.45	18.75	18.25	17.25	18.0

Designations of every division of bodies of worms employed in Table 24 are the same as those used in Table 17 and all the figures given here were obtained by experimenting with the same parts of three different individuals at the same time.

H. M. VERNON (1899) said, " The temperature of onset of heat contraction and of loss of excitability do not seem to depend at all on the amount of solid constituents in the tissues." (p. 286.) However, in his observation, from which he deduced the above mentioned conclusion, he attempted to compare the onset heat-shortening temperature and solid constituents in muscles belonging to very widely different cold-blooded animals, but he did not determine the relation between the two factors in question in muscles belonging to the same animal. Hence, his statement cannot be accepted bodily without further test on the question just pointed out.

(2) T. H. MORGAN and A. C. DIMON (1904) found that in two species of earthworm, Lumbricus terrestris and Allolobophora foetida the anterior and posterior end, in general, were electronegative, galvanometrically, to the middle. The result of their work, generally speaking, was coincident with that of the writer's heat-shortening experiments in Allolobophora foetida and Allolobophora caliginosa (see Charts 19 and 20), though their work was only qualitatively done. Therefore, it may be said to be possible that the portion, which needs a higher temperature to produce heat shortening, is electronegative to those of a lower onset temperature. Regarding such a relation I cited the data of mine.

TABLE 25.

Exper.	Heat Rigor	Electrical Polarity		
Cond. of Temp		16°–18°	15°–19°	±0.5°
Season	Summer	Winter	Summer	
Unit.	Degree C	Milli Volt		
A	40.2	-11.2	-9.6	-5.0
B	39.7	5.3	-6.5	-3.7
C	38.9		-4.8	-2.7
D	39.2	1.8	-2.0	-0.4
E	38.6		0.9	+1.6
F	38.0	2.9	+0.6	+2.9
G	38.4		+0.5	+2.5
H	38.4	0.0	0.0	0.0

In the above table, the figures in the first column are cited from the foregoing Table 17; those in the second column, from my previous work (1928), p. 144, Table III. The figures in the third and fourth columns are given from the writer's recent observations in 1927, which are not yet published. The data in these two columns indicate the average values of the results of experiments on twenty-five worms, and the measurement of the electrical potential has been carried out twice in every worm on the dorsal and ventral sides of the body. The methods of measurement and the experimental conditions have already been published (1928) and therefore the readers are referred to the writer's previous work concerning this subject. The experiment, of which the result is shown in the last column, has been done with the purpose of observing the effect of cooling upon the gradient of the electrical potential.

The following chart shows in brief, intimate correlations among the gradient of the heat-shortening temperature, the gradient of the amount of solid constituents and the gradient of the electrical potential in the earthworm, *Pheretima communissima*.
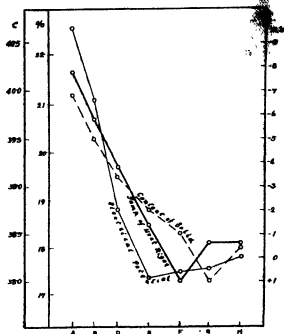
Chart 31. Gradient of content of solid constituents, gradient of
temperature of heat rigor (heat-shortening), and gradient of electrical
potential in the earthworm, *Pheretima communissima*. These graphs
are based on the data given in third and fourth columns of Table 23
and those given in the third column of Table 25

It is generally observed that embryonal tissues in vertebrates
are much richer in water content than the corresponding tissues of
the adult, and as development goes on, their content of water generally
decreases through the storing up of the formative substance, which is
equivalent to stating that the degree of differentiation of the tissues
or organs is reversely related to the water content.[1] Accordingly,
the content of water, that is, the content of solid in a reverse sense,
may be assumed as a chemical sign of differentiation or senescence in
tissues, and the gradient of the content of water or the content of
solid constituents may represent that of the tissue differentiation.

The researches of CHILD and his associates seemed to prove, in

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[1] DAVENPORT (1908) Chapter X, pp 281–292; MORGAN (1907) Chapter XV, pp.
239–252; ROBERTSON (1923) Chapter X. pp. 260–272.

many species of animals, that the metabolic gradient is closely correlated with the electrical potential gradient. Regarding this correlation he said (1924), "In general, in animals the higher levels, i.e., the levels of more intense activity, of higher rate of oxydation, are electronegative, galvanometrically, to lower levels." (pp. 87–88).[1] Therefore, it may be said that the gradient of the electrical potential represents that of the metabolic rate, and my own observations showed that the electrical potential gradient is correlated with the heat-shortening gradient, so far as the earthworm is concerned.

On comparing the facts mentioned above, it seems highly probable that the heat-shortening gradient is also associated closely, with that of tissue differentiation as well as that of metabolic activity, though not directly. To say these apparant correlations, existing between all gradients so far studied, indicate true causal physiological connections among each other we need far more evidence that we have now from different angles.

As already known in general, for a long time, the heat rigor is not true death, but a state of temporary rigidity and a tetanus condition of the tissues, from which tissues may be return to a normal condition of life when the temperature is not raised above this point and is shifted towards the optimum under favourable conditions. Death is likely to occur at a few degrees above the temperature of this rigid state, and beyond this point recovery is impossible. So the state of heat rigor is not an eternally non-recoverable break-down of vital activity, but is merely a temporary standstill of it. If it be so, the heat-shortening gradient indicates that in the animal body such a maximal temperature-limit of metabolic activity in tissues differs according to the different parts of the body and varies in a specific manner in each species of worm along the axis of the body. So this gradient seems to signify differences of metabolic activity in different parts of the body, in relation to the habitats or behaviors of the animals under not yet unveiled influences.

Here, in connection with the heat-rigor gradient, we are reminded of Nysten's law concerning the rigor mortis of the human body. According to this law, after the complete cessation of heart beating,

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[1] In the recent works of E. J. Lund (1928 a, b), the relation between electrical potential and tissue respiration has been accurately studied.

rigor mortis occurs first in the head muscles, next in the neck muscles and then in the muscles of the trunk and extremities in sequence downwards in the order of the segmentation of the spinal chord; that is, NYSTEN's law, so to speak, indicates the axial gradient of rigor mortis in the human body, which may be another expression of the phenomenon of heat rigor, or heat shortening.

   According to E. GOTSHLICH (1893), the heat rigor is an incomplete and transitional stage of rigor mortis. From this viewpoint, heat rigor and rigor mortis are merely two cases, which appear with some quantitative difference, in a continual series of temperature effects in the contractile tissues, and according to this writer there is no exact point at which the heat rigor occurs   This last statement may be true theoretically, but in practice the determination of the onset point of heat shortening can easily be detected with any amount of exactness needed. Indeed, according to the imbibition theory of rigor, which has been rather recently advanced by MEIGS, and VON FÜRTH and LENK, heat rigor is the phenomenon caused by the imbibition of water by tissue substance due to the accumulation of a large amount of lactic acid explosively produced there on heating. If this is the real case, difference in the onset temperature of heat shortening in tissues may be directly attributed to the physico-chemical conditions of the colloidal behavior of tissue constituents; in other words the heat-shortening gradient may be construed as one of the simplest and most direct expressions of the difference of the physico-chemical constructions of tissues along the axis of the animal body.

                           SUMMARY

   1. In chaetopod annelids the onset temperature of initial heat-shortening is different in different parts of the body or body wall and varies in the form of a *gradient* along the axis of the body.

   2. Among the forms of heat-shortening gradients in chaetopods, there are four types. Two of them are known respectively as the monopolar and bipolar types or the primary and secondary types of chaetopod gradients, while the other two are quite different from the former two and I have named them the third and fourth types, respectively. The third type of gradient resembles an inverted image

of the bipolar type, that is, in this type the onset temperature is highest at about the middle part and decreases gradually towards both extremities of the body, having one maximum and two minima. In the fourth type, the onset temperature is also highest at about the middle part and gradually decreases towards both extremities of the body, but near the posterior end is lowest than the anal segments and increases again at the very end, so that the fourth type is more complex than the others, having two maxima and two minima.

3. The four types of heat-shortening gradients were noted and each type was found to be specific and characteristic for each species. Although in general the different groups of chaetopods show a characteristic type of gradient, owing to the presence of exceptional cases, the causal connection of different types of gradient with phylogenetic advance of chaetopods can not be too strongly emphasized until the heat-shortening gradients in embryonic or younger larval stages are examined with respect to possible transformations of the gradient types during growth.

4. The heat-shortening gradient is closely correlated with those of the content of solid constituents and the electrical potential, the second of which in turn, is, according to other invertigators, related to metabolic activity.

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Notes on the Development of a Holothurian, Caudina chilensis (J. Müller).[1]

By

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(With Plates IX–XIV and 13 text-figures)

The present work deals with the development of *Caudina chilensis* (J. MÜLLER), an apodous Holothurian belonging to the family Molpadiidae, starting from the egg and tracing up to the larva of about 1 mm. length. It was carried out during the last two years of 1928 and 1929, while I was staying at the Marine Biological Station at Asamushi.

Most of the work, such as the artificial fertilization of eggs, the rearing and feeding of the larvae and the observations upon them, etc., was executed chiefly in the first year and from this a general idea was obtained of the course of development of this animal, while in the second year the results thus obtained in the first year were reexamined and also some gaps which were left in the same were filled.

It is generally known that there exist three types in the mode of development in Holothurians.

In the first type the typical Auricularia stage occurs as reported by MECHNIKOFF (1869), SELENKA (1883), SEMON (1888) and BURY (1869 and 1895) in the case of *Labidoplex (Synapta) digitata*, by SELENKA (1876) in *Holothuria tubulosa* and by others in other forms.

The second type has not the Auricularia stage in the course of development and thus the larva is transferred directly to the Doliolaria stage as in the cases of *Cucumaria echinata* (OHSHIMA 1918, 1921), *Cu. frondosa* (RUNNSTRÖM 1919), *Cu. planci* (KOWALEVSKY 1867, SELENKA 1876, LUDWIG 1891) and other Cucumarians.

The third type is that recorded by H. L. CLARK in 1898 in the case of *Synapta vivipara (Synaptula hydriformis)*. The larva of this

form is quite distinct from both the Auricularia larva in the first type and the Doliolaria larva in the second in being deprived of ciliary bands or even of any trace of cilia.

All the forms which hitherto served as the material of the embryological studies in Holothurians were the members belonging to the three families only, namely Cucumariidae (Dendrochirotae), Holothuriidae (Aspidochirotae) and Synaptidae.

In regard to the family Molpadiidae which includes the apodous forms among Actinopoda none of the embryological works have been hitherto performed. This fact is due, I think, to the difficulties in securing suitable materials.

It was quite fortunate that I was able to observe on the development of a member of this interesting group, having had the opportunity to be endowed with a plentiful supply of materials. Thus I could ascertain that it belongs to the second type of development above alluded to, and passes through the Doliolaria stage during the metamorphosis.

I wish to express my sincere thanks to Professor S. HATAI under whose suggestion this interesting work was undertaken. To Professor S. HOZAWA and to Professor H. OHSHIMA of the Kyushu Imperial University my hearty thanks are due for their kind advices and very helpful critisisms given me during the course of the work. I am also grateful to Assistant Professor S. KOKUBO of the Marine Biological Station at Asamushi who gave much time to help me in my studies.

MATERIAL AND METHOD.

The materials which served the present investigation were chiefly obtained at Moura, a village about 3 miles distant from the Marine Biological Station at Asamushi, where *Caudina chilensis* is found very abundantly.

To secure eggs and larvae of the early stages and thus to determine the breeding season of this animal a plankton-net with a mouth of 0.3 meters diameter was hauled once or twice every week. It was tried during the high tide both close to the sea surface and to the bottom.

To obtain sea-water and estimate quantitatively the eggs or larvae

contained in it, a hand pump was used.

Also a dredge of small size was employed for the collection of the larvae of more advanced stages which live burrowed in the bottom sand.

In addition to the above the materials were also secured by artificial fertilization of eggs in the laboratory. The eggs thus far manipulated were reared in either small beakers or in glass dishes. These beakers or dishes were tightly covered with glass plates and were soaked in a larger glass vessel filled with running sea-water, with the hope to keep the temperature of the feeding media as constant as possible. In the experiment of 1929, the temperature of the feeding media thus far obtained was nearly constant showing 13.7°±0.5°C., for the first ten days of observation. During that period the fertilized ova developed up to larvae of about 1 mm. length.

For the killing and fixing eggs and larvae FLEMMING's chromo-aceto-osmic mixture and the sublimate-formol were used. NAWASHIN's chromo-aceto-formol mixture gave good results in fixing the material for the observation on chromosomes. To cut the section, both the paraffin method and the celloiden-paraffin were employed. Most of the sections were cut of a thickness of 5-6 μ and only in a few cases, such as to observe the chromosomes in egg, they were cut into 8 μ thicknesses.

To stain the sections HEIDENHEIN's haematoxylin and organe-G were used combined and the safranin and EHRLICH's haematoxylin were also tried and were found able to obtain good results.

The graphical method was used for the reconstruction

BREEDING SEASON

To define the breeding season of this animal the investigation of the plankton was tried and thus the eggs were first noticed on May 21st, 1928 and on May 29th, 1929. Here I would like to mention that the eggs as well as the larvae of *Caudina chilensis* are easily distinguished from those of others on account of their brownish-red colour (Pl. IX, fig. 1). .

In 1928 either eggs or larvae were noticeable every day during the period extending from May 21 up to June 15, while it was from

May 29 up to June 28 in 1929. It was also observed that after the
said period there appeared neither eggs nor larvae in the plankton-net.

In the sea-water taken by the hand pump the eggs were found
once on May 21, 1928 and the larvae also once on May 31 of the
same year.

The results obtained from the observation of the plankton are
mentioned in more detail in the following table.

TABLE I.

| Date (1928) | Eggs or larvae of *Caudina chilensis* obtained by | | | |
| | Plankton-net | | Hand Pump | |
	Occurrence	Stage	No. of eggs or larvae in 30 lits. of sea-water	Stage
Before May 12	—	—	0	—
May 12	—	—	0	—
15	—	—	0	—
21	+ c	E	9	E
23	+ r	G	0	—
24	+	E G	—	—
26	+	B E	—	—
27	+	B E	—	—
28	+	B E G. L.	—	—
29	+ c	B. G. L.	—	—
31	+	B G. L.	3	B. L.
June 6	—	—	0	—
8	+ r	G.	0	—
11	—	—	0	—
15	rr	L	0	—
21	—	—	0	—
24	—	—	0	—
After June 24	—	—	0	—

B	Blastula	c.	Concentrate
E	Egg	r.	Rare
G	Gastrula	rr	Very Rare
L	Larva		

Judging from the facts obtained from the observations made on the larvae hauled by the plankton-net at different times of the day, the spawning of this animal seems to take place naturally during the period of high tide which follows the low tide occurring in day time.

TABLE II.

Date (1928)	Time at which the plankton-net was hauled.	Stage of eggs or larvae	Time of high tide[1] in the second period	
			beginning	highest
May 21	3. p. m	unsegmented egg	11. a m	5. p m
23	9. a m	blastula	11.30 a m	6 p. m
	5.30 p m	gastrula		
24	6. a m.	later gastrula	1.40 p m	7. p m
	3. p. m	eggs of unsegmented or 4-cell stage		
	1.30 a m.	blastula		
26	5. a m.	blastula	3.30 p. m	10. p m.
	8.30 a m.	blastula		
	noon	active blastula		
	11. p. m	eggs of about 16-cell stage		
27	8. a. m.	blastula	4. p. m	10 p m.
	2. p. m.	active blastula		
	6. p m	eggs of 2- or 4-cell stage		
28	8. a. m.	gastrula	4. p. m	11.30 p m
	1. p. m.	gastrula		
	7. p m	later gastrula & unsegmented egg		

On the other hand the observations on the spawning habit of this animal also were made during the breeding season. For this purpose some full-grown animals representing males and females were kept in

[1] The time of high tide is referred to the record given by the tide-gauge at Kukurisaka near Asamushi.

a glass vessel[1]. They were collected at Moura, above mentioned. The modes of shedding off the genital elements are nearly the same in both sexes, keeping their bodies and tentacles entirely quiet. The spawning lasts usually for half an hour but it is prolonged, rarely, up to three hours. I have had the chance to observe the phenomenon nine times, i. e. six times in 1928 and three in 1929 and the most of them occurred in the evening not later that about 10 o'clock.

From these facts above alluded to it may be concluded that the breeding season of this animal, in the vicinity of Asamushi, begins about the middle of May and extends up to about the last day of June, and it is also recognized that the spawning occurs most frequently during the period extending from late May to early June, when the temperature of sea-water is measured at 13.5°–18°C

GENITAL ORGANS

Genital papilla. — A short conical genital papilla measured 4.5–8.0 mm. in length is situated near the base of the tentacular crown along the mid-dorsal line and it is of yellow tint in the adults. This papilla is lacking in a specimen smaller than 100 mm. long. When the spawning comes to an end, the tip of the papilla looks red in colour in both sexes, probably owing to the congestion of the red blood in it. But this colour disappears gradually and within two or three days it recovers the ordinary colour.

Female gonad. — In full-grown individuals the ovarian tubules are transparent and are yellowish-brown in colour containing numerous dark-brown eggs of various sizes and thus they may be easily distinguished with the naked eye from the male gonads, to be mentioned later on. The full-grown ovarian egg is slightly flattened and is attached by its broad surface to the wall of the ovarian tubule or to that of the blood vessel of the ovary. It contains a large quantity of cytoplasma, and a large and spherical germinal vesicle (nucleus) (Pl. X, figs. 6, 7, 8, 9, N) carrying many germinal spots (nucleori) (Pl. X, figs. 6, 7, 8, 9, no). The germinal vesicle lies in the early stages of development in the centre of the egg cell, while in the later

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[1] This manipulation is the so called "live-box method" and was used by SELENKA, EDWARDS, OHSHIMA and others in obtaining the larvae of the Holothurian.

stages it is eccentric, sitting near the free end of the same (Pl. X, fig. 9). The number of the germinal spots is gradually decreased changing in number from about 10 into 2-3 after the increase of size of the egg (Pl. X, figs. 6, 7, 8, 9).

The intravitelline membrane (Pl. X, figs. 6, 7, 8, IV) is recognizable in all stages of immature eggs as in the case of *Caudina arenata* (GEROULD, 9, Pl. 6). When the egg grows and attains a diameter of 0.2 mm. or more a minute conical process of cytoplasma, called the micropyle appendage (OHSHIMA, 26, p. 183), begins to appear at the centre of the free surface of the egg (Pl. X, fig. 8, ma). This appendage becomes more distinct when the eggs are more grown and when they have the gelatinous space between the egg membrane and the follicular epithelium (Pl. X, fig. 9, ma). GEROULD (9, Pl. 6, figs. 88, 89) has observed this peculiar structure at the earliest stage of the immature egg in *Caudina arenata*, but in the case of *Cucumaria echinata*, OHSHIMA (26, Pl. 8, fig. 3) has observed it in the full-grown eggs only.

Male gonad. — In the breeding season the genital tubules of the male are filled with ripe spermatozoa and appear yellowish white in colour. In the cross-section of the ripe male gonad, some large cells are to be found close to the germinal epithelium, they may be probably assumed as spermatocytes (Pl. X, fig. 12, spc). These cells measure 0.008-0.01 mm. in length and their large oval nuclei are 0.004-0.006 mm. in diameter.

The features of a spermatozoon are the same as those of other Holothurians figured by JOURDAN, FIELD and RETZIUS. In Jod-Jod-kalium preparations the spermatozoon shows a small round acrosome at the anterior portion of the head and a crescentic middle piece at the posterior-most portion of it. The head is 0.004-0.005 mm. long. The spermatozoon also has a long tail, measuring 0.04-0.06 mm. in length.

The fully matured spermatozoa are to be seen through the season extending from the middle of September to late May. They are all spawned at one shedding when the breeding season arrives. The same phenomenon was also observed in the case of *Caudina arenata* (GEROULD, 9, p. 179) and of *Stichopus japonicus* (MITSUKURI, 20, p. 7).

FERTILIZATION AND MATURATION.

The eggs which are spawned will float on the surface of sea-water when its specific gravity is 1.0228 at 15°C. and 1.024 at 4°C. The weight of a single egg is calculated at about 51×10^{-6} grams. Each of the eggs is ellipsoidal in shape being slightly flattened in the animal pole, as in the cases of *Cucumaria echinata* (OHSHIMA, **26**, Pl. 8, fig. 4) and of *Cu. normanii* (NEWTH, **24**, Pl. 1, fig. 1). The average length of each of the three axes measured in 15 specimens is 0.5718 mm., 0.4920 mm. and 0.3855 mm respectively The eggs are naturally shed when the eggs arrive at the stage of metaphase of the first maturation division (Pl X, fig 10) as in the case of *Thyone briareus* reported by OHSHIMA (**27**, Tab. I, fig. 5).

The chromosome number of this species has been estimated to be fourteen in the heterotypic (Pl. X, fig. 11).

Fertilization. — When the spermatic fluid is added to the sea-water which contains the floating unfertilized eggs there occurs, probably, fertilization, and the eggs soon sink to the bottom, but in a short time they float again as before or are suspended in the feeding sea-water. In such eggs the fertilization membrane may be clearly observed around the egg body leaving a transparent layer 0.06–0.07 mm. thick.

Maturation. — The maturation division will take place after the fertilization was performed and thus the extrusion of the first polar body occurs within about one hour as in the cases of *Leptosynapta inhaerens* (RUNNSTRÖM, **30**) and of *Thyone briareus* (OHSHIMA, **28**). The second polar body is constricted off within half an hour after the extrusion of the first polar body and then the first polar body is divided into two thus presenting three polar bodyies in all (Text-fig. 1, c. pb).

GENERAL EMBRYOLOGY.

a. Cleavage.

The cleavage is total and approximately equal as in the cases of other Holothurians, however, in most cases the eggs are divided irregularly and only in a few cases they are segmented in a radial manner when observed at the early period reaching up to 32-cell

stage. The first cleavage begins in about two hours, then the second occurs within 30-50 minutes following the first. The first two cleavage planes are usually meridional, crossing perpendicularly and thus the egg is divided into four approximately equal blastomeres (Text-fig. 1. a). The third cleavage is equatorial and thus the egg bears eight blastomeres, however, in most cases the blastomeres are not formed simultaneously (Text-fig. 1. b) and then there appear often some eggs consisting of three or six blastomeres. When the cleavage has advanced and when the egg has passed its 8-cell stage, the blastomeres begin to show to some extent a spiral arrangement (Text-fig. 1. c). The succeeding segmentations after the third could not be traced clearly but it seems to be sure that they are not strictly regular as in the cases of *Cucumaria saxicola* and *Cu. normanii* (NEWTH, 24).

The blastula is of a solid type, its blastocoele containing some numbers of cells which were formed by the proliferation of the blastoderm cells (Text-fig. 2).
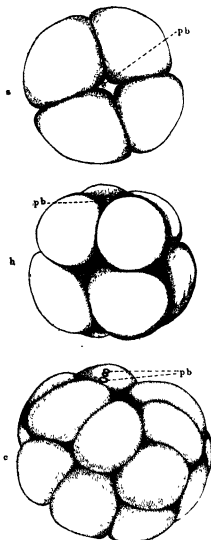
Text-fig. 1. Early stages of the egg of *Caudina chilensis* in segmentation. ×100. a. 4-cell stage; b. about 8-cell stage; c. the fourth cleavage at the 4th hour. pb = polar body.
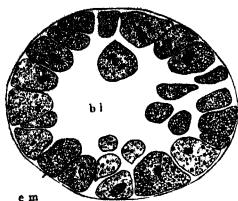
Text fig 2　Cross section of th　arly t las ula
of *Caudina chilensis* at the 8th hour　×150
bl=blastocoele　em　egg membrane

At about the 10th hour a number of the wrinkles which are so-called egression tracts (Text fig 3 4 5 etc) begin to appear on the surface of the blastula. They correspond in number to the folds formed by the invagination of the blastoderm Each of the folds first appears as a small intercellular space

among the blastoderm cells (Text fig 4 a) The egression tracts increase in number from 3 to 18 with the advancing stages of development of the egg The most characterist c features of the wrinkled blastula are observable at about the 19th hour (Text figs 3 a b 5 b) Some of these tracts are divided at both ends and appear in the manner of a figure X be ng measured about 0 4 mm long and 0 13 mm deep (Text fig 3 a) Finally the tracts will be decreased in their number in accordance with the closure of the folds at their bases (Text fig 4 d) Thus the superficial tracts disappear and the surface of the blastoderm becomes smooth again at about the 24 hour stage (Text fig 5 c) and at that time it is observable that the cells of the blastoderm increase in number and are arranged in five or six layers. The larva reached at the stage above mentioned acquires cilia 0 018-0.02 mm long placed all over the blastoderm A few of the f lds which were seen in the wrinkled blastula often remained in the blastocoele (Text fig 6 1 each as a small mass enclosing a lumen inside until about the two or three day stage

At the stage of the 29th hour the larva emerges from the egg membrane rotating slowly along its long axis mostly in a clockwise manner The larva which has just emerged from the egg membrane is slightly smaller than the egg measuring 0 51 mm long and 0.42 mm wide
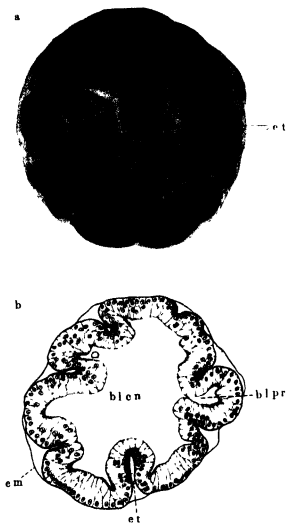
Text-fig 3. The wrinkled blastula of *Caudina chilensis* at
the 19th hour × 150
    a. external view.
    b. median cross section.
blcn = central blastocoele, blpr = peripheral blastocoele, em = egg-
membrane, et = egression tract.
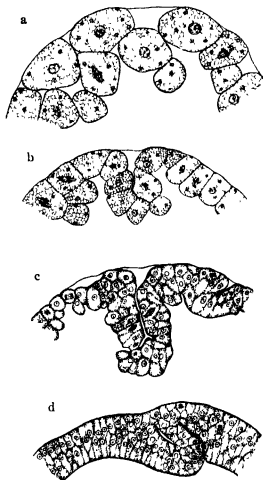
Text fig 4 Showing the changes of one of the folds
occurring inside of the wrinkled blastula of *Caudina chilensis*
×150
a at the 10th hour, b at the 12th hour, c at the 19th hour,
d at the 22nd hour

Immediately before the gastrulation some number of free mesen-
chyme cells are proliferated at the vegetative pole and are placed
close to the latter in the blastocoele (Text-fig 6, me)
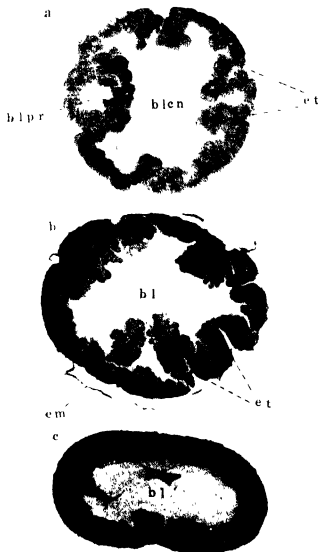
Text-fig. 5   Sections of wrinkled blastula of *Caudina chilensis*, showing the inside. ×95

a. at the 12th hour; b. at the 19th hour, c. at the 24th hour.

bl.=blastocoele, blcn=central blastocoele, blpr=peripheral blastocoele, em=egg-membrane, et=egression tract

c. Gastrulation and Dipleurula [1]

At about the 33rd hour, gastrulation takes place and the invagi-
nation first appears at the vegetative pole (Text-fig. 6, a). The

archenteron thus far pro-
duced gradually increases
in length in accordance
with the growth of the
larva Text-fig 6, b). At
the end of the second day,
the archenteron attains a
length longer than the half
of that larva, and its pos-
terior half begins to bend
toward one direction. In
this stage, some cells at the
anterior end of the archen-
teron are liberated into the
lumen of the latter and they
are destined to be future
blood corpuscles Text-fig. 7,
(c) Then the archenteron
is twisted and is constricted
into two parts, the flat ex-
panded hydro enterocoele is
anterior and the tubular gut
is posterior (Pl. XI, figs, 13,
17, hye, g) Then the gut
is separated from the hydro-
enterocoele within 3 4 hours
and grows anteriorly taking
the sinistrorose direction.

Text-fig 6 The gastrula of *Caudina chi-
lensis*
a median section of the gastrula at the 33rd
hour ×95
b median section of the gastrula bearing a
straight archenteron at the 47th hour ×75
ar - archenteron, bl blastocoele, blp -blasto-
pore, me mesenchyme cell

The hydro-enterocoele
begins to be constricted
transversely at a level a
little beyond the middle.

[1] OHSHIMA, 25, p 380, 26, p 199

The anterior part of the hydro-enterocoele thus formed grows towards the right side, while the posterior one which is destined to be the future enterocoeles is going to be divided into two parts, right and left as its dorso-posterior end The anterior part of the hydro enterocoele grows ventrally and is destined to be the future hydrocoele, and it also gives rise to a number of lobes to become future tentacles at its dorsal side (Pl XI, fig 19) Then the hydrocoele which carries four lobes of the future tentacle and rudiments of radial water canals is separated from the enterocoele (Pl XII, fig 24) Of the future tentacles, the first two lie on the right side, the third which is smaller than the others lies at about the mid dorsal point, and the fourth on the left side (Pl XII fig 24)

The posterior part of the hydro-enterocoele alluded to is thus divided into the right and left vesicles before it is separated from the hydrocoele. The antero dorsal ends of both the right and left vesicle come close and meet at the mid dorsal line while the remaining parts of both grow larger and surround the gut

On the other hand, the stomodaeum begins to appear as a depression of the ectoderm at about the middle of the ventral side (Pl XI, figs. 21, 22; Pl XII, fig 26, st)

This stage corresponds to that called by OHSHIMA " Dipleurula " in the case of Cucumaria echinata (25, p 380, 26, p 199) But in
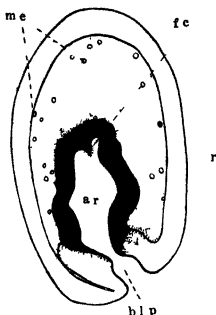
Text-fig 7 The median frontal section of the gastrula of *Caudina chilensis* to show the cells which are proliferated from the cells located at the anter or end of the archenteron and which give rise to bloo l corp iscles ×150 ar archenteron blp~llastocoele fc—blood corpuscle me mesenchyme cells

the present species it is not clearly distinguishable from the next
Doliolaria stage in the features of ciliation

Such larvae swim immediately below the surface of the sea and
their size as measured in the living state is about 0 62 mm long and
0 43 mm wide

d   Ciliation of the Ectoderm

At the end of the second day the surface of the larva is uniformly
covered with cilia and this state is retained even after the larva has
emerged from the egg membrane until about the 48th hour (Text fig
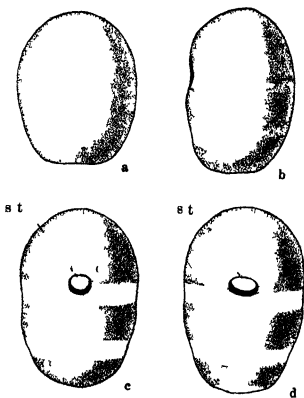
Text fig 8 Showing the mode of formation of ciliary band in the
larva of *Caudina chilensis* ×100
a c, d the larva viewed from the ventral side   b the same viewed
from the left side. st = stomodaeum

8, a). Then the uniform arrangement of cilia will be disturbed. This disturbance first occurs at the ventral side and afterwards spreads towards the dorsal (Text-fig. 8, b). At about the 55th hour, one or very rarely two transverse ciliary bands appear surrounding the middle of the body (Text-fig. 8, c). Thus the cilia occurring on the surface of the larva are now grouped into three parts, i.e. those of the preoral lobe, those forming a ciliary band and those of the posterior end of the body. When the larva is more grown the cilia occurring at the posterior end of the body are arranged in a transverse band surrounding the anal field (Text-fig. 8, d; Pl. IX, fig. 1), while those of the preoral lobe gradually disappear in accordance with the growth of the stomodaeum. The cilia begin to degenerate when the larva has attained the Pentactula stage and thus the locomotion of the larva becomes gradually inactive and finally it sinks to the bottom.

e. Doliolaria.

The larva of this stage shows the characteristic external features appearing like a barrel. It is reached at about the 72nd hour and lasts until the fifth day. At the beginning of this stage, a syncytium of the ventral ectodermal plate (Pl. XII, figs. 26, 30, ve) is observed surrounding the stomodaeum and facing the blastocoele. Some of the cells forming this plate are differentiated into the cells which construct the central nerve ring (Pl. XIII, figs. 34, 35, nv) while the remaining cells are transferred into the ectodermal cells of the tentacles. Each of the radial nerves which bears a semicircular epineural canal, and which arises from the nerve ring above mentioned runs posteriorly along the outside of each radial water canal (Pl. XIII, figs. 35, 36, 37, nr 1–5). At the end of this stage, the radial nerve is shorter than the radial water canal and thus the posterior end of the former does not yet reach to that of the latter (Pl. XIII, figs. 37, 38).

The differentiation of the hydrocoele begins at the end of the third day and thus it is divided into the following components, (Text-fig. 9; Pl. XII, fig. 28) i.e., the ring canal (Text-figs. 9, 10, ri), five primary tentacles of which 'one is rudimental, one closed pore canal (Pl. XII, figs. 28, 29, 30; Text-fig. 9, pc) which is to be transferred into the stone-canal in future, one lobe-like structure destined to be

the future Polian vesicle (Text-fig. 9, Pv) and five rudimental radial water canals. Here it will be explained more precisely about these components. The canal which is destined to be the future ring canal is not yet completed as to form an entire ring but presents a shape like the figure C. If it is divided by the mid-dorsal line into two halfs of right and left, the right half bears two primary tentacles and two rudimental radial water canals, while the left half, which is larger than the right, carries three primary tentacles, one lobe to become the future Polian vesicle and three lobes of rudimental radial water canals. If the five primary tentacles are demonstrated as to their position, the first is right ventral, the second is right dorsal, the third is left dorsal lying close to the pore canal, the fourth is also left dorsal but occurring more ventrally than the third and the fifth is much smaller than the others and forms the left terminal portion of the ring canal together with the rudimental Polian vesicle and the mid-ventral radial water canal.

d

v

Text-fig. 9 Diagram show-ing the principal organs to be seen in the early Doliolaria stage in *Caudina chilensis*
d = dorsal side, enl = left entero-coele, enr = right enterocoele, g = gut, pc = pore canal, Pv = Polian vesicle, rcm = mid-ventral radial water canal, r₁–₅ = ring canal, t₁–₅ = primary tentacles, v = ventral side

Of the five radial water canals, the fifth, lying mid-ventrally, was refered to above, while the remaining four are still small and lobe-like (Text-fig. 9, rcm).

The free ends of the ring canal approach nearer and at last come to contact with each other at a point a little right of the mid-ventral line, and at the same time the fifth tentacle, which was formerly small, now grows as large as the others. These five primary tentacles above alluded to begin to turn their tips towards the atrial cavity (Pl. XIII, fig. 33; Pl. XIV, fig. 39, at). When this stage is advanced these tentacles are attached to the ring canal in such a manner as mentioned below (Text-fig. 10). The first and fifth are situated at each side of the mid-ventral water canal, their lumen being com-

municated with that of the latter (Pl. XIII, fig. 35). The third and the fourth occur at each side of the léft dorsal radial water canal and their lumen communicates with that of the latter (Pl. XIII, fig. 34). Lastly the second is placed at the base of the right dorsal radial water canal, its lumen being communicated with that of the latter. Thus no tentacles are set in connection with the left ventral radial water canals as well as the right ventral of the same (Pl. XIII, fig. 32).

The pore canal is not yet opened to the exterior at the beginning of this stage (Pl. XII, fig. 28). But on the fifth day it grows dorsally and is communicated to the exterior by means of a dorsal pore (Pl. XIII, fig. 37).

The lobe of the rudimental Polian vesicle develops into a pear-shaped Polian vesicle (Text-fig. 10, Pv) and is attached to the inner side of the ring canal between the fourth and the fifth tentacles.

The two enterocoeles which were first separated from the hydrocoele develop ventrally with their ends approaching to meet at a short distance from the mid-ventral line in the left and thus they surround the gut at last. Of the enterocoeles the ventral end of the left comes inside of the right and the outer wall of the former is set in contact with the inner wall of the latter (Text-fig. 10). Of these contacted walls fusion occurs only in the anterior-most portion, while the rest remain as the ventral mesentery. The dorsal ends of both entero-coeles also come into contact with each other and thus the dorsal mesentery is formed. The dorsal mesentery (Pl. XIV, fig. 44, mt) holds the anterior part of the gut, being attached along the mid-dorsal line of the latter, and the ventral one holds the posterior part of the gut which coils once before reaching the anus.

The stomodaeum grows inwards and its posterior end reaches to the anterior end of the gut. Its opening to the exterior is situated at first between the preoral lobe and the ciliary band at a point slightly left of the mid-ventral line, as in the cases of *Cu. echinata* (OHSHIMA, 25, 26) and *Cu. normanii* (NEWTH, 24). At the end of this stage the stomodaeum is situated at the lower part of the preoral lobe. At the end of the third day, the gut runs in nearly the same manner as in the adult. It runs at first posteriorly reaching to the half of the enterocoele, then turns forwards attaining the anterior-most part

of the enterocoele, then again runs towards the end of the body terminating at the anus.

The calcareous deposits begin to appear at the later part of this stage.

The larvae of the later Doliolaria stage above dealt with were obtained in most cases by means of a plankton-net

f. Pentactula

The larva attains to this stage in the sixth day and it was observed to be retained until about the twentieth day (Pl IX, fig 1, Pl. XIV, fig 39).

The five radial nerves increase their length and their extremities reach to the end of the radial water canal (Pl. XIV, fig. 45).

Up to this stage, the tips of the tentacles were thickly covered with ectoderm and were simple, but at the end of the sixth day each of them is divided into two digits (Pl. XIV, fig. 39). The five primary tentacles which thus bear two digits are seen to be protruded to the exterior through the atrial cavity and again to be with-drawn.

The radial water canals are more grown and their tips reach to the posterior end of the body.

The madreporic ve-

Text-fig 10 Diagram showing the principal organs to be seen in the Pentactula stage in *Caudina chilensis*

d = dorsal side, enl = left enterocoele, enr = right enterocoele, g = gut, pc = pore-canal, Pv = Polian vesicle, rcm = mid-ventral radial water canal, ri = ring canal, t₁₋₅ = primary tentacles, v = ventral side.

sicle which first arose from the pore-canal near its base, now has increased in size and is destined to become the future madreporic body (Pl XIV, fig. 39, 45, md)

The dorsal pore which communicated with the exterior at the end of the preceding Doliolaria stage is closed again at this stage at about the end of the ninth day, before the larva begins to sink to the bottom of the sea burrowing in the sand.

The enterocoele and the gut do not show any characteristic changes to be specially demonstrated during the present stage

In accordance with the growth of the body and with the decrease in size of the preoral lobe (Pl XIV, fig 39, Prl), the stomodaeum is removed anteriorly in position and finally it comes to the anterior end of the body as shown in the cases of *Cucumaria frondosa* and *Psolus phantapus* (J & S RUNNSTROM **29**, Text fig 25)

At about the ninth day, the pentactula larvae begin to sink to the bottom of the sea and to creep by means of their tentacles The larva of this stage measures about 0.913 mm long and 0.493 mm wide

Judging from the results obtained from the experiment, it is highly probable that the larva burrows into bottom sand on about the tenth day and the larvae are retained in the same stage for ten more days in the sand

Here it is necessary to mention the fact that I was not able to find any trace of pedicels or even of the pedicel canal through all the larval stages of the present species

The Pentactula larvae, together with larvae of the preceding stages, are opaque and are brownish red in colour as seen in the case of the egg

g   Young

The feeding of the young was continued from the 25th of May until the 20th of July in 1928 and from the 30th of May until the 2nd of August in 1929. But I was not able to obtain the young of more advanced stage than that which bears four secondary tentacles in addition to the five primary tentacles And the larvae thus far reared are smaller in size than those naturally produced The young which were collected from the sea bottom at about the middle of June were all those bearing the fifteen tentacles, each of which

carried four digits at its tip Of these four digits, two are primary
while the remaining two are secondary which were produced at the
base of the first (Text-fig 11, dt_1, dt_2).
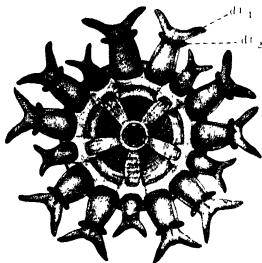
Text-fig 11   Frontal view of the mouth parts of the
young of *Caudina chilensis*, showing the formation of the
secondary digits (dt_2) at the base of the primary (dt_1) × 30

The integument of the young is translucent and thus most of the
internal structures are observable through it from the outside.   The
brownish-red tint which was seen in the larval stages is retained only
in the alimentary canal.

GROWTH IN THE FIRST YEAR

When the young is burried in the sand it swallows the sand within
its intestine in great abundance and the body is thickly clothed with
sand grains all over its surface (Pl IX, fig 4).   As it is very difficult
to remove these sand grains both inside and outside of the body, the
weight of the young could not be measured, though the volume was
determined by measuring its diameter[1] and length[2] under a fully

---

[1] The diameter is measured along the widest part of the body
[2] The length is measured by the distance from the base of the tentacles to the
proximal end of the tail

expanded condition in the sea-water.

MITSUKURI (**20**, pp. 13–16) applied the formula length × breadth² to determine the approximate volume of *Stichopus japonicus*, and EDWARDS (**8**, p. 239) used the formula $\frac{\pi l r^2}{2}$ in the cases of *Holothuria arta* and *H. floridana* for the same purpose. The shape of the animals of these species, however, differs from that of *Caudina chilensis*, being destitute of tail portion. So I have applied in the case of the present species the formula of an ellipsoid, $V = 4/3 \pi$ abc or $4/3 \pi$ ($\frac{1}{2}$ diameter)² × $\frac{1}{2}$ length
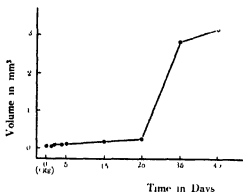
Text-fig 12   Curve to show the growth of the young of *Caudina chilensis*, which were reared by the artificial fertilization of the egg

Though the young which were reared in the laboratory room were much smaller than those reared naturally in the sea, they presented the data given in the Table III. concerning their volume.

To measure the volume of animals grown under natural conditions, specimens were collected at the same spot in Moura Bay at intervals of ten days during the period extending from the 11th of June to the 29th of November of 1928. The results thus far obtained are shown in Table IV. and in Text-fig. 13.

Recently YAMANOUCHI reported a formula Ws = 0.03422 L to represent the relation between the body length and the weight in the case of the present species. In this formula L is body length in cm. and Ws represents the body weight including the sand within the intestine

TABLE III.

Age in days	No of animals	Average length (L) in mm	Average diameter (2r) in mm.	Volume calculated (4/3πr² × ¼ L) in mm³
Egg[1]	15	—	—	0.053
29 hrs.	2	0 51	0.42	0.047
2	1	0.62	0.43	0.069
4	2	0.71	0.48	0.087
5	1	0.87	0 47	0.101
15	5	0.96	0.61	0.187
25	4	1.28	0 56	0.210
35	2	2.40	1.50	2.827
45	2	3.05	1 40	3.130

(34, p. 338; p. 346). But it must be remembered that the formula
is only applicable to the young not heavier than 32 grams. I, too,
have measured the weight of this animal using the formula given by
YAMANOUCHI and obtained the results also shown in Table IV. and
Text-fig. 13.

As it is clear from the table and text-figure, these two results
seem to coincide pretty well and equally show that the animal grows
rapidly when it passes the stage in which all of the fifteen tentacles
carrying four digits are completed.

TABLE IV.

Date	No. of animals	Average length (L) in mm	Average diameter (2r) in mm	Volume calculated (4/3πr² × ¼ L) in mm³	Weight calculated (Ws = 0.03422 L³) in milligrams
June 11	13	1.91	0.90	0.81	0.24
21	8	3.41	1.52	4.11	1.35
July 2	11	4.43	1.90	4.47	2.97
11	19	5.61	1.71	8.46	6.02
21	28	9.21	2.89	39.87	26.71
31	24	11.08	3.62	79.11	55.75
Aug. 10	40	13.22	3.65	91.61	79.00
20	35	11.36	3.26	84.67	64.63
Sept 9	50	13.02	3.90	103.68	75.58
20	30	11.71	3.79	87.71	55.06
Oct 11	35	13.98	4.47	149.70	92.43
21	17	16.38	4.52	176.30	150.14
Nov. 6	15	14.25	4.15	127.62	98.84
29	24	18.56	5.07	248.77	218.54

[1]The volume of the egg is calculated by the same formula measuring its three
    axes
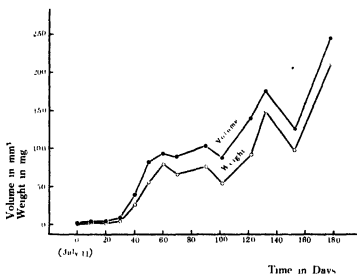
Text-fig 13. Curves to show the growth of the young of *Caudina chilensis* which were obtained at Moura

DISCUSSION.

The egg which sinks in the feeding medium of sea-water, was reported in *Holothuria floridana* (EDWARDS, 16), *H. tubulosa* (SELENKA, 31), *Cucumaria echinata* (OHSHIMA, 25, 26), *Cu. kirchsbergii* (KOWALEVSKY, 12), *Thynone briareus* (OHSHIMA, 28) and that which floats on the same was reported in *Cu. planci* (SELENKA, 32), *Cu. frondosa* and *Psolus phantapus* (RONNSTRÖM, 29). The specific gravity of the egg of *Caudina chilensis* is about 1.024 and thus it floats on the surface of sea-water which has the salinity greater than 30.9‰. The larvae of Holothurians are mostly obtainable either by the live-box method or by taking them out directly from the coelom or the brood cavity of the mother animal in the viviparous habit. And thus they differ from Asteroidea and Echinoidea, the larvae of which are easily reared by artificial fertilization. In Holothurians the artificial fertilization was successful only in a few cases such as reported by MORTENSEN in *Holothuria nigra* (22), *H. sp.*, *Stichopus kefersteinii* and *St. californicus* (23) and by COURTNEY (5) also in *St. californicus*, while many other investigators, however, did not succeed in performing the

same. In the present species both the artificial fertilization and the
live-box method were equally successful, when a fully ripened egg was
obtainable in the former case.

The cleavage in the Holothurian egg, except in a case of *Cucu-maria glasialis* (MORTENSEN, 21) which is segmented superficial, is
generally total and is mostly equal and radial. In this species it is
total but is of a very irregular type and thus it makes it very difficult
to trace the mode of segmentation even in eggs which have passed
only a small number of cleavages.

In regard to the wrinkled blastula it was described by GEMMIL
(10) in two Asteroids of *Solaster* and *Porania*, and by MASTERMAN
(19) in an Asteroid, *Cribrella oculata* In Holothuroidea it was obser-
ved in the cases of *Cucumaria normani* and *Cu. saxicora* by NEWTH
(24). A similar wrinkled stage was reported also by DES ARTS (6)
in *Cu. frondosa* under abnormal conditions, but it was denied by
RUNNSTROM in the case of the same species. All the species which
pass the wrinkled stage in the course of their development are those
which pass the solid morula stage before coming to the wrinkled stage.
But in the case of *Caudina chilensis* it comes directly to the wrinkled
stage, passing the solid blastula stage instead of the solid morula.

The larvae of *Caudina chilensis* emerge from the egg-membrane
before the gastrulation begins as in the cases of Cucumarids and thus
it differs from *Leptosynapta inhaerens* (RUNNSTRÖM, 30) and *Holothuria
frondosa* (EDWARDS, 7). In these two species the gastrulation begins
within the egg-membrane.

In *Caudina chilensis*, the formation of the free mesenchyme cells
precedes the gastrulation as in the cases of *Cu. frondosa* (RUNNSTRÖM,
29), *Cu. echinata* (OHSHIMA, 25), *Cu. planci* (LUDWIG, 16) and *Psolus
phantapus* (RUNNSTRÖM, 29). In Synaptids, the formation of the
mesenchyme cells takes place at the tip of the archenteron as in
Labidoplax digitata (SELENKA, 32), *Leptosynapta inhaerens* (RUNNSTRÖM,
30) and *Synapta vivipara* (CLARK, 2). In *Holothuria frondosa* (ED-
WARDS, 7) and *H. tubulosa* (SELENKA, 31) the mesenchyme cells are
formed at the time when the gastrulation begins. Concerning these
facts LUDWIG (16, p. 258) reported that the difference of the time at
which the mesenchyme cells are formed is due to the difference in
the velocity with which the development proceeds. Regarding the

same problem OHSHIMA (26, p. 194) mentioned that " in those forms
where the mesenchyme formation takes place early the cells are gene-
rally very numerous and they readily fill up the blastocoele, while in
those where invagination precedes the mesenchyme formation the cells
are generally few ". To these reasons above alluded to I may add
that the difference is also based on the specific characters bearing on
the phylogenetical meaning.

In *Caudina chilensis* the manner of the development of the archen-
teron in the gastrula stage and these of the hydrocoele and the gut
in the Dipeurula stage are almost the same as those seen in the cases
of Cucumariidae, such as *Cu. echinata* (OHSHIMA, 25, 26), *Cu. normani*
(NEWTH, 24), *Cu. planci* (LUDWIG, 16), *Cu. frondosa*, *Psolus phantapus*
(RUNNSTRÖM, 29) and also in the cases of Synaptiidae, as *Leptosynapta
inhaerens* (RUNNSTROM, 30).

In *Caudina chilensis* the differentiation of the enterocoele begins
earlier than in any cases of other Holothurians such as *Cu. echinata*
(OHSHIMA, 26, p. 203) and *Cu. planci* (SELENKA, 31, p. 171), and it
is separated into the right and the left enterocoele before it is sepa-
rated from the hydrocoele.

In most species of Holothurioidea it is known that the ciliation in
the gastrula stage is nearly uniform, and that the Doliolaria larva
usually bears a number of ciliary bands. But in the case of *Cu.
normanii* reported by NEWTH (24, p. 634) the ciliation was uniform
not only in the gastrula stage but also in the Doriolaria. In the case
of *Synapta digitata* these features are quite different. The gastrula
of this form is entirely deprived of cilia and the Doliolaría also bears
no trace of cilia. In the case of *Caudina chilensis*, it is noticed that
the Doliolaria carries only a single ciliary band. In the case of *Cu.
echinata*, OHSHIMA (25, 26) has recorded three ciliary bands in the
doliolaria and in the case of *Cu. planci* four ciliary bands were reported
by KOWALÉVSKY (12), LUDWIG (16) and SELENKA (31, 32).

In the early Doliolaria stage, of *Caudina chilensis*, three primary
tentacles of the five are arranged on the left half of the ring canal
while the remaining two are situated on the right half of the same.
These features are just the same as those seen in the cases of *Cu.
frondosa* (RUNNSTRÖM, 29), *Holothuria floridana* (EDWARDS, 7), *Labido-
plax digitata* (SEMON, 33), *Leptosynapta inhaerens* (RUNNSTRÖM, 30)

and *Synapta vivipara* (CLARK, 2). But when the Doliolaria larva is
more grown and when the ring canal is completed, forming the entire
circle, these primary tentacles are arranged in a manner almost like
that seen in the cases of *Cu. echinata* (OHSHIMA, 25, 26), *Cu. frondosa*
(RUNNSTRÖM, 29), *Cu. planci* (LUDWIG, 16) and *Psolus phantapus*
(RUNNSTRÖM, 29), but different from that in *H. tuburosa* (SELENKA, 31).

The type of development which passes through a typical Auricularia
stage was described by J. MÜLLER (1848)[1], METSCHINIKOFF (1869)[2]
and SELENKA (1883, 32) in the case of *Synapta digitata*, by SELENKA
(1876, 31) in *H. tubulosa*, by MORTENSEN (1913, 32; 1921, 23) in *H.
nigra*, *H. sp* and *St. californicus* and by COURTNEY (1927, 5) in *St.
californicus*. It is observed that these forms all belong either to the
family Holothuriidae or the family Synaptiidae.

The type of development in which the larva directly attains the
Doliolaria stage without passing the Auricularia was reported for the
first time by DANIELSSEN and KOREN (1859)[3] in *Cu. frondosa* and
later by KOWALEVSKY (1867, 12), SELENKA (1876, 31; 1883, 32), and
LUDWIG (1891, 16) in *Cu. planci*, by NEWTH (1916, 24) in *Cu. normanii*
and *Cu. saxicola*, by OHSHIMA (1918, 25; 1921, 26; 1925, 28) in *Cu.
echinata* and *Thyone briareus*, and by RUNNSTRÖM (1919, 29; 1928,
30) in *Cu. frondosa*, *Psolus phantapus* and *Leptosynapta inhaerens*.
These forms are all members of the family Cucumariidae except
Leptosynapta inhaerens which belongs to the family Synaptiidae.

In regard to the family Molpadiidae, the type of development
seems to remain undetermined under such state as reported by H.
L. CLARK (3, p. 152) " Nothing whatever is known of the embryology
of the Molpadiidae, save that GEROULD (1896, 9) has studied the ooge-
nesis and to some extent the spermatogenesis of *Caudina arenata* ".

By the facts obtained from the present investigation we are able
to ascertain that *Caudina chilensis*, a member of the family Molpa-
diidae, belongs to the type of development which was first reported
by DANIELSSEN and KOREN in the case of *Cucumaria frondosa* and
thus it passes through the Doliolaria stage instead of the Auricularia.

It is highly assumable that other forms of the present family will
also take the course not far different from that of *Caudina chilensis*
in their development.

SUMMARY.

The results obtained from the observations on the development of *Caudina chilensis* are summerized as follows.

1. The breeding season begins, in the vicinity of Asamushi, in the middle part of May and ends in late June.

2. The spawning of the genital products takes place during the high tide which succeeds the low tide occurred during the day-time.

3. The artificial fertilization of the egg was easily and successfully performed.

4. The egg is ellipsoidal in form, measuring about 0.57 mm. long, about 0.49 mm. broad and about 0.39 mm. high. The specific gravity is nearly 1.024. It is opaque and brownish-red in colour and these features make the egg easily distinguishable from those of other Holothurians.

5. The eggs are laid during the metaphase of their first matura-tion division which will take place within about one hour after fertili-zation.

6. The cleavage of the egg is total but is of irregular type and the first cleavage occurs within two hours after fertilization.

7. The blastula is of solid type and in its blastocoele a number of cells which proliferated from the blastoderm cells are observed.

8. The characteristic wrinkled blastula is clearly observed.

9. The gastrulation first appears at the vegetal pole at about the 33rd hour. The archenteron is first twisted in the sinistrorse direction and finally is devided into three parts which are called respectively hydrocoele, enterocoele and gut.

10. The Doliolaria stage is noticeable at the end of the third day and the Pentactula stage first appears in the sixth day.

11. Only one ciliary band exists, surrounding the middle of the body, while the cilia occur in mass on the preoral lobe and on the anal field.

12. Five primary tentacles are given to rise at about the 72nd hour and afterwards their tips are projected into the atrial cavity at about the 100th hour. The tip of each tentacle is divided into two digits at the end of the sixth day. Two other digits, i. e. the secondary digits, are formed at the base of the primary digits when all the

fifteen tentacles are completely arranged, at about the middle of July.

13. The radial water canals begin to appear at about the 72nd hour and each of their posterior ends reaches up to the end of the body on the sixth day. However, the posterior end of each radial water canal remains simple and is not divided at this stage as in the adults.

14. The pore-canal is first noticeable at about the end of the third day and it opens to the exterior on the dorsal surface along the median line at about the 100th hour.

15. The madrepolic vesicle is first recognized at about the sixth day attaching to the base of the pore-canal which arises from the ring canal.

16. The nervous system is derived at first from the ventral ectodermal plate at about the third day. The nerve ring is observed around the anterior part of the gut and at the base of the tentacles at the end of the fifth day. At the same time it is recognizable that the five radial nerves arise from the nerve ring and run along the outer side of the radial water canal.

17. The right and left enterocoeles form the coelomic cavity, their ends being set in contact with each other and forming the dorsal and ventral mesenteries.

18. At the end of the third day the Doliolaria has the gut which first runs posteriorly and then anteriorly and lastly again posteriorly making a coiled loop, and terminates in the anus which lies at the posterior extremity of the body.

19. The eggs as well as the larvae of the various stages up to the Pentactula were obtainable by means of a plankton-net.

20. The young of this animal seems to grow more quickly when it has passed the stage in which all its fifteen tentacles are completely arranged.

21. Judging from the facts above demonstrated, it may be concluded that the present species, though it is deprived of pedicels, presents some closer affinities to Cucumaridae than to Holothuridae or Synaptidae in view of the type of its development.

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EXPLANATION OF THE PLATES

LIST OF ABBREVIATIONS

an = anus ar = archenteron. bl = atrial cavity. bl = blastocoele blcn = central blastocoele blp = blastopore. blpr = peripheral blastocoele ' cv = coelomic cavity. cvp = peripharyngeal part of the coelomic cavity. d = dorsal side. dp = dorsal pore. dt₁ = primary digit of the tentacle. dt₂ = secondary digit of the tentacle em = egg membrane. en = enterocoele. enr = right enterocoele. enl = left enterocoele epc = circular epineural canal. epr = radial epineural canal. et = egression tract ' fc = blood corpuscle or feed cell of the archenteron. fl = follicular epithelium. g = gut. hy = hydrocoele. hye = hydro-enterocoele iv = intravitelline mem-

brane. lbv=lacunar blood vessel of the ovary. l=left side. ma=micropyle appendage. md=madreporic vesicle or axial sinus mc=mesenchyme cell msg=circular muscle of the germinal tubule. mst=muscle of the tentacle. mt= mesentery. nv=nerve ring. nr=radial nerve. nr_{1-5}=radial nerve N=nucleus or germinal vesicle. no=nucleolus or germinal spot. pc=pore-canal. pb_1= polar body. prh=preoral hood. prl=preoral lobe Pv=Polian vesicle r=right side. ri=ring canal. rc=radial water canal. rcm=mid-ventral radial water canal. rcld=left dorsal radial water canal. relv=left ventral radial water canal rcrd=right dorsal radial water canal. rcrv=right ventral radial water canal spc=spermatocyte spz=spermatozoa. st=stomodaeum sy=syncytium t= primary tentacle. t_1=primary tentacle in right ventral interradius. t_2=the same in right dorsal interradius t_3=the same in mid-dorsal interradius t_4=the same in left dorsal interradius t_5=the same in left ventral interradius. v=ventral side   ve=ventral ectodermal plate   w=wall of the germinal tubules

PLATE IX.

Fig. 1   The Pentactula stage of *Caudina chilensis* (J MÜLLER), viewed from
          the ventral side. ×75.

Fig. 2   The young with seven tentacles, about ten days after it has burrowed
          in sand, viewed from the right side ×35

Fig. 3   The same with nine tentacles, ten days after it has passed the stage
          mentioned in fig 2, viewed from ventral side ×35

Fig. 4   The same with fifteen tentacles obtained at Moura on July, 16 in 1928.
          It is thickly clothed with sand grains. ×75

Fig 5   The same, from which the sand grains were removed, showing the
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PLATE X.

Fig. 6.   The very young ovarian egg ×450

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Fig. 26. The 64th cross section, cut along the plane 64 in fig 24 ×110.

Fig. 27. The 80th cross-section, cut along the plane 80 in fig 24 ×110

Fig. 28. Early Doholaria stage at the 72nd hour, viewed from the right side;
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Fig 29. The 62nd cross-section, cut along the plane 62 in fig 28, ×110.

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Fig. 32 Later Doholaria at the 100th hour, viewed from the right side; recon-
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Fig. 44. The 57th cross-section, cut along the plane 57 in fig. 39. ×150.

Fig. 45. The median longitudinal section of the same stage ×150.

1   3   2
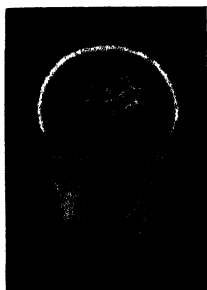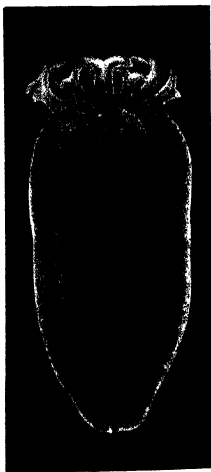
4   5

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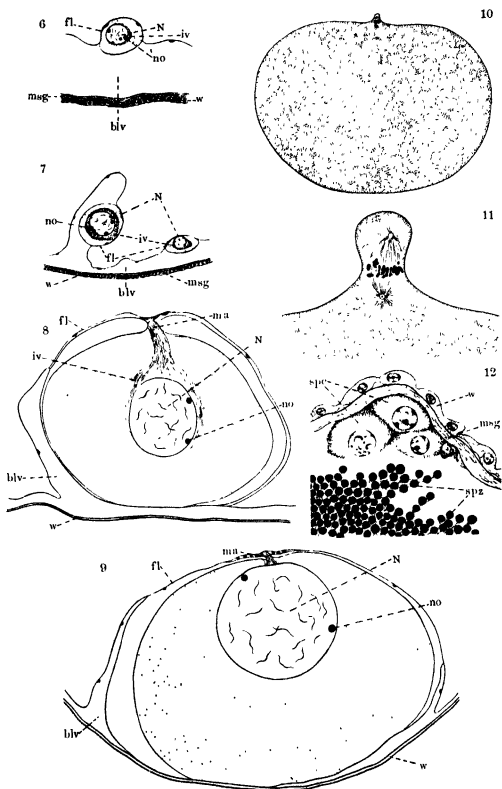
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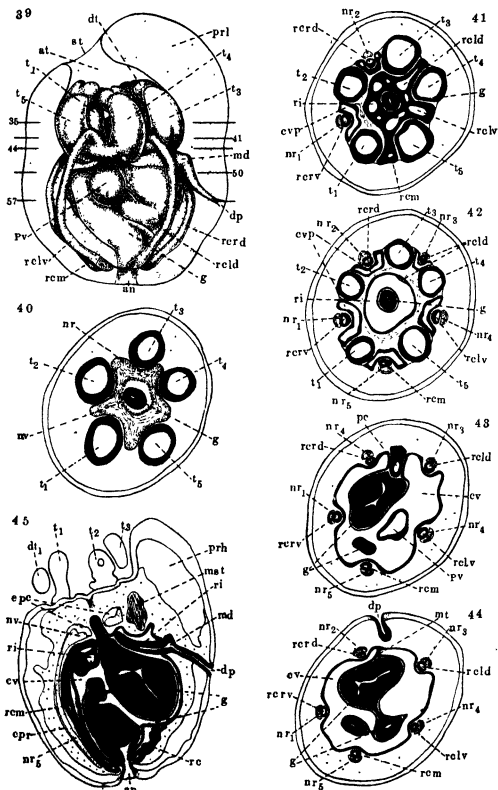
D. INABA: Development of *Caudina chilensis* (J. MÜLLER).

Contribution to the Research on the Respiration of Fishes

II Studies on the Acidosis of Fishes [1]

By

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(25 text figures)

CONTENTS

INTRODUCTION

In the first publication (44) under the above general title the pH and the CO_2-content of the blood of fishes such as *Cyprinus*, *Leuciscus*, and several other marine animals were described. The work has been extended with a view to observe whether these characters show any response to altered conditions of the external medium.

Most aquatic animals have their blood in close contact with the external medium, i.e. surrounding water, though the blood and water are separated from each other by a delicate gill membrane. Therefore one would anticipate a disturbance of equilibrium between blood and water associated with a change in the composition of breathing water. It is thus, of physiological interest to observe the behaviour of the blood of aquatic animals toward the external medium, and likewise of biological significance when this phenomenon is seen from the standpoint of comparative physiology. DAKIN (15), SUMNER (3) (39), and GREEN (21) focussed their interest on this point in regard to the study of the osmotic relation of the blood. The present author (27) also studied the response of Oyster blood to the altered condition of sea water.

To determine whether aquatic animals exhibit the change of acid base equilibrium with the change of reaction or other ingredient of the breathing water, similar to those observed in land animals, the present investigation was undertaken. It is for this reason that I have first paid my attention to the problem of acidosis.

The term acidosis was first introduced by NAUNYN (33) in 1906 to mean simply the abnormal occurence of beta-oxybutyric acid and aceton in the blood of diabetes. After that, however, it was found that there are many acidosises resulting from quite different origins which can not be regarded as mere acid intoxication. For instance, diabetic acidosis means the evolvement of organic acid in the blood, while nephritic acidosis refers to the lowering of the CO_2 tension in alveolar air. Moreover the diminution of alveolar CO_2 in man by removal to a high altitude was regarded as a kind of acidosis. Furthermore, the decrease of plasma CO due to anesthesia was likewise regarded as an acidosis.

Thus the cause of the acidosis seems to be very varied, but all acidosis has the common characteristic that it decreases the alkali reserve, i. e. the CO content of the blood.

The commonest acidosis which come to our attention can be enumerated as follows.

(1) Diabetic acidosis Owing to the production of beta-oxybutyric and other acids in the blood, the alkali reserve of blood decreases.

(2) Nephritic acidosis. As the acid accumulates in the blood on account of the defect of kidney, the alkali reserve of the blood diminishes and consequently the alveolar CO_2, decreases.

(3) High altitude acidosis. By a forced respiration or over ventilation due to relative decrease of O_2 tension in atmospheric air, the blood loses an excessive amount of CO_2, thus decreasing the alkali reserve.

(4) Anesthesia acidosis. Through excessive evaporation of CO_2 by hyperpnoea, the alkali reserve of the blood decreases.

(5) Experimental acidosis. By injection or administration of acid the blood lowers its alkali reserve.

Besides the above examples, there are several kinds of acidosis which are producible by fasting, anoxaemia, cardiac or gastric diseases etc.

Thus nowadays the term acidosis means even merely a shift of acid base balance of the blood. Consequently NAUNYN's acidosis is only a minor part of acidosis and is called under the name ketosis.

It is well known that VAN SLYKE (40) classified acid base balance in 9 orders, by low, high, or normal of the alkali reserve and pH of the blood. Recently CULLEN and AUSTIN (4) proposed to call VAN SLYKE's uncompensated acidosis, i. e. severe acidosis accompanied by an abnormally low pH and low alkali reserve, "true acidosis". The British National Research Council proposed to understand the term acidosis as meaning the high and low levels of blood alkali, while the terms alkalemia and acidemia were as meaning the high and low pH of the blood (AUSTIN and CULLEN, 4).

Besides the term acidosis I have however used the term alkalosis, which is in use by many other authors in contrast with the term acidosis, meaning a shifting of the acid base balance of the blood to the alkali side of the normal value. Alkalosis is nothing else than extreme conditions of the acid base balance, i. e. VAN SLYKE's uncompensated alkali excess (high pH and high alkali reserve) and uncompensated CO_2 deficit (high pH and low CO_2 tension), of which the former condition corresponds to the term alkalemia above mentioned.

Alkalosis is produced for instance, by hyperventilation, hindrance of alkali excretion, alkali injection, alkali administration etc.

The present investigation is a study of acidosis and alkalosis of fishes following the alteration of the pH of breathing water. In addition to this, the effect of the anoxaemia on the acidosis was also observed.

ACKNOWLEDGEMENT

CHAPTER I.

GENERAL PART 1. METHODS USED.

I METHOD OF EXPERIMENT.

1) Experimental acidosis and alkalosis. Acidosis and alkalosis in higher animals, such as mammals, may be produced by several experimental methods: (1) By a dose of some excess of acid (HCl, H_3PO_4, NaH_2PO_4, $C_3H_6O_3$) or alkali ($NaHCO_3$, Na_2HPO_4), (2) By an injection of acid or alkali into the blood circulation, and finally (3) Alkalosis from a voluntary hyperpnoea or a forced dyspnoea, and acidosis from an anoxaemia. Besides these methods, an ingestion of NH_4Cl or $CaCl_2$ (AUSTIN and CULLEN, 4, 8), Cantharidin, Arsenous acid, and other chemical substances (GOTO, 20), salvarsan or trypaflavin may also produce acidosis. A diabetic acidosis may be brought about by removing the spleen from a dog (GOTO, 20). The impairment of the renal function due to nephrectomy highly disturbs the acid base equilibrium of the blood, so that an apparent acidosis occurs (AUSTIN and CULLEN (4)). A meal causes first a rise and then a fall of alveolar CO_2 tension, indicating the change of the acid base balance of the blood (16) (30), and, moreover, a fasting for some duration also causes acidosis Further, the use of anaesthesia (14) (28) produces the acidosis. Therefore all these causes enumerated above may be applied in inducing acidosis experimentally. Whether or not all these methods are equally successfully applicable to the case of fishes must await future investigation. In the present investigation I have employed the same method which was used in producing acidosis and alkalosis in oyster (27) by merely changing the pH of the breathing water, or by decreasing O_2 tension in breathing water.

2) Arrangement of Experiment. The essential part of the experimental process consisted in that the fishes were forced to respire for a definite time in the acid or alkali water. In each experiment one to several fishes were introduced in a jar measuring 35 cm in diameter and 40 cm in height. The breathing water used was almost 20 liters in all cases. As the O_2 quantity of the breathing water diminishes due to respiration of fishes the water was aerated in some cases, and not in others. During the course of the experiment the fishes were

frequently bled at varied intervals.

3) *Water temperature.* In the experiment of *Cyprinus* the brea-thing water was warmed in most cases, because the tap water was too cold at the time of experiment. In the experiments made on *Cyprinus* the lowest temperature sank to 3.6°C, and the highest tem-perature rose to 26.3°C. But most experiments were made at 11° to 25°C. In the experiments conducted on *Leuciscus* the breathing water was not warmed, since the lowest temperature did not fall below 10° and the highest temperature did not rise above 14°C. Most experi-ments were made at 11° to 14°C and furthermore each experiment was carried out with the least possible temperature fluctuation.

4) *Duration of experiment.* The duration of experiment varied with the object of the experimentation In the experiment on *Cyprinus* the duration ranged from 3 to 72 hours. In the first 4 experiments in which the recovery of the acidosis was tested, the duration extended to as long as 24 to 48 hours While in experiments 5-10 the water temperature was raised in order to accelerate the effect of experimental condition, so that the duration was shortened to from 3 to 5 hours. The alkalosis experiment ranged from 24 to 72 hours, in view of determining the time of recovery.

As regards *Leuciscus*, most experiment were finished within 2 to 4 hours, except two cases, one of which needed only 40 minutes while the other continued for 48 hours In as much as acidosis takes place in *Leuciscus* very rapidly, there was no need of continuing the experi-ment longer.

5) *Regulation of the pH of breathing water.* The pH of the breathing water was kept considerably high in one case or low in the other by adding either the solution of NaOH or HCl. Since the production of CO_2 by the fishes tend to lower the high pH of water, while the low pH water showed a perpetual tendency to raise its pH by the neutralization of acid by fishes, it was necessary to add acid or alkali at a definite rate in order to keep constant the initial pH of the breathing water. For this purpose I employed an automatic dropper (Fig. 1) which was made by modifying the ordinary aspiration bottle. This dropper has a content of about 600 cc, and from 5 to 50 drops p. m. of alkali or acid solution can be dropped as desired by regulating a stop-cock beneath the bottom.

The solution of NaOH or HCl used was $\frac{n}{5}$ or $\frac{n}{10}$ in most cases, though the $\frac{n}{1}$ solution was also sometimes used. When the fishes were disposed to breath in a dilute buffer solution the pH of the water kept constant for a long while. But in such solution the fishes became much weakened after a while by the intoxication due to the buffer solution. For instance, in a *Cyprinus* which was forced to respire in the 20 fold diluted solution of ordinary citrate buffer solution, the cortex of the eye turned opaque after 2 hours, and the fish became very inactive. In addition, the breathing water was contaminated and become frothy, showing a very unsuitable appearance for the experiment. Therefore in my present investigation the buffer solution was not used.

6) *Conditions for observation.* During the experiment the following eight conditions were observed and recorded. (1) Air temperature, (2) Water temperature, (3) pH of the breathing water, (4) pH of the blood, (5) CO_2-content of the blood, (6) Frequency of respiratory movement, (7) Behaviour of fishes, (8) O_2 content of the breathing water.
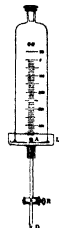
Fig 1. Automatic dropper.

A — aspirating hole.
L — levelling dish.
R — regulating cock.
D — dropping tip

II COLLECTION OF BLOOD.

For the blood taking much skill and experience were needed, inasmuch as the fishes had to be bled repeatedly so as not to weaken them until the end of the experiment. As all the bleeding was done by heart puncture it was necessary to orientate the exact position of the heart in the body from without. In *Cyprinus* the heart is situated at the middle of the line which connects the posterior end of the basal part of both pectoral fins. But in *Leuciscus* it is situated at a slightly more anterior as compared with *Cyprinus*. The heart, however, is rather small in bulk, so the insertion of the syringe needle must be made very carefully. The syringe needle was about 0.7 mm in

thickness and 4 cm in length, and was provided with a pipette. The pippette measured 1 cc and was graduated to hundredths. Prior to the bleeding the pipette was filled with paraffin oil, and after puncturing this needle into the heart the pipette was sucked with the mouth by a rubber tube which was attached to the distal end of this pipette, thus preventing the contact of the blood with the air. By this method 1 cc of blood can be obtained within 1 minute. Moreover, as is well known, the clotting of the fish blood takes place very rapidly, so that a bleeding must be finished within 1.5 minutes at the longest.

As soon as the blood was collected the pH and CO-content were determined within 5 minutes. The quantity of the blood required for each determination varied with the fish. In *Cyprinus* 0.5 and 0.1 cc were used for the determination of the pH and CO_2-content respectively, while in *Leuciscus* 0.25 and 0.1 cc were used, for the same purpose Consequently the total volume of the blood used for each determination was 0.5 cc in *Cyprinus* and 0.35 cc in *Leuciscus*, but the real amount of blood collected each time was about 1 cc in *Cyprinus* and 0.5 cc in *Leuciscus*.

Although the frequency of bleeding throughout the entire course of experiment, varied according to fishes it was 3 to 12 times in *Cyprinus*, and 2 to 8 times in *Leuciscus*.

The number of fishes employed for an experiment was 1 to 6 in the case of *Cyprinus*, and 3 to 5 in the case of *Leuciscus*. The total determinations which were made in one experiment numbered 33 times in *Cyprinus* (Alkalosis Exp. 5.) and 28 times in *Leuciscus* (Acidosis Exp. 10) at the most

III. MATERIALS

The fish used as material were the following two species.

Cyprinus carpio LINNE.

Leuciscus hakuensis GÜTHER.

The reason why I preferred the above was, first of all, the ease of collection of these two species. The former being a most commonly cultured fish in Japan, it was at our disposal throughout the year. *Leuciscus* is also a common sea fish which is regulary taken in abundance in May every Year in the neighbourhood of our station. In

addition to this convenience, both are robust fishes and are of moderate size, and consequently are able to provide a sufficiet quantity of blood. Moreover, the former being a fresh water fish, while the latter is a sea inhabitant, we may consider them as representatives of fresh and sea water fishes.

The total number of fishes used was 48 of *Cyprinus* and 50 of *Leuciscus*. Sizes of the *Cyprinus* varied from 28 to 36 cm in length with an average length of 30.8 cm, and weighed 321 to 882 gms, with a mean weight of 520 gms. *Leuciscus* measured 33.1 to 48.0 cm in length with a mean of 35.8 cm, and weighed 333 to 890 gms in weight with a mean of 506 gms.

IV   DETERMINATION OF THE BLOOD pH

For the determination of the blood pH CULLEN's (13) colorimetric method which was employed in my previous investigation (26) was again adopted. According to AUSTIN, STADIE, and ROBINSON (3) the colorimetric reading of the blood changes continuously when it is diluted at an increasing rate by the standard saline solution, notwithstanding CULLEN's statment that the dilution of 20 fold gives fairly a constant value.   Therefore it happens that the result obtained by CULLEN's method in which the colour is read under 20 fold dilution, regarding this dilution as optimal, does not represent the constant value.   In other words the dilutions in the neighbourhood of 20 fold never give any constant value, but the pH changes directly with the degree of the dilution.

Disregarding the human blood, which is the subject of the above argument, the fish blood changes its colour reading too intensely to use any dilution as optimal. That is to say, the change of the colour reading due to dilution in fish blood is by far greater than of the human blood (26). And hence to obtain the true pH value of fish blood by CULLEN's method a difference of the electrometrical pH and the colorimetrical pH read under a definite dilution must be previously determined. If this correction is made the colorimetric pH of the fish blood is readily converted into the true pH.

Therefore in a study in which the relative change of the pH is mainly discussed, the colorimetric method is by far the best means,

because of its simplicity and the economy of time and material. In my present investigation the pH determination had to be made as frequently as possible within a limited time. For this reason, I again preferred CULLEN's method. The application of this method was made in a similar manner to that of the former investigation, and the pH was determined under 11 fold dilution in *Cyprinus* and 21 fold in *Leuciscus*.

Redistilled Water

The standard saline solution used for the present method required a high sensitivity for alkali which may be produced by the blood. To serve this purpose the distilled water used for the preparation of this solution was redistilled, and even the trace of alkali was eliminated. The redistilled water prepared in our laboratory was highly sensitive to the addition of alkali or CO_2, and fully served my purpose. The sensitivity of the redistilled water was tested in the following way.

5 cc of redistilled water was taken in a hard-glass test tube, adding 0.25 cc of phenol red (0.02%). The color of the water thus produced showed a yellow tone due to the acidity of CO_2 already contained. When this test tube was heated by a strong burner the water began to boil within 30 seconds. As the water became alkaline by the evaporation of CO_2, the colour began to change within 1 minute. After continuing the boiling 3 minutes, the test tube was taken out from the flame and tightly stopped with a ruber stopper. The pH was then determined immediately before cooling. But, as the phenol red rapidly alters its colour with the temperature, the pH was once more read after it cooled to 20°C ·

In this examination, however, much care must be taken in the choice of the test tube, for if the tube is not hard enough, the result will be wholly disturbed by the alkali evolved from the glass.

For the purpose of comparison, a like examination was also made on ordinary distilled water and tap water. The results of 12 determinations made on a sample were listed in the Table 1.

From an inspection of the above table one will notice that the tap water and distilled water contain much more alkali than the re-distilled water. Accordingly the highest sensitivity of redistilled water

TABLE 1.

No.	Redistilled water			Distilled water			Tap water		
	Before heating	During heating	After cooling	Before heating	During heating	After cooling	Before heating	During heating	After cooling
1	Below 6.80	6.80	7.84	Below 6.80	7.80	7.90	7.00	8.30	Above 8.60
2	,,	6.90	7.85	,,	7.90	8.00	,,	8.30	,,
3	,,	6.87	7.90	,,	7.35	7.85	,,	8.25	,,
4	,,	6.90	7.34	,,	7.90	8.00	,,	8.30	,,
5	,,	6.95	7.35	,,	7.29	8.00	,,	8.35	,,
6	,,	6.85	7.36	,,	7.35	7.95	,,	8.35	,,
7	,,	6.90	7.83	,,	7.35	7.90	,,	8.30	,,
8	,,	6.90	7.35	,,	7.29	8.00	,,	8.32	.
9	,,	6.87	7.30	,,	7.33	7.95	,,	8.35	.
10	,,	6.85	7.35	,	7.35	7.97	,,	8.34	,,
11	,,	6.86	7.34	,,	7.35	7.90	,,	8.32	,,
12	,,	6.87	7.35	,,	7.35	7.90	,,	8.30	,,
Mean	,,	6.88	7.54	,,	7.37	7.94	,,	8.32	,,

will result from the least quantity of alkali contained because the sensitivity of water to the change of pH may be diminished by this alkali, making a buffer system combined with the carbonic acid

V. DETERMINATION OF THE CO_2-CONTENT OF BLOOD.

The determination of the CO_2-content of blood accompanied in all cases the determination of the blood pH. Accordingly this determination had also to be made several times in one experiment. The apparatus used was a volumetric micro-apparatus of VAN SLYKE

This apparatus needed 0.1 cc of the blood and about 5 minutes of time for each determination. In looking for complete accuracy, the manometric apparatus of VAN SLYKE (43) which was employed in my previous work (29) was desirable, but for the sake of economy of time a volumetric apparatus was preferred.

VI. BREATHING WATER.

For the breathing of *Cyprinus* fresh water was used, while for *Leuciscus* both fresh and sea water were employed.

1) *Fresh water*. The fresh water used was the tap water of the laboratory. The pH of this water ranged between 7.10 and 7.60 in

fresh condition, though it fell to pH 6.80 when it was exposed to the
atmospheric air for a long while. No special analysis has been made
to determine the bicarbonate content of this water. But it is
surmised to be very rich in bicarbonate, for the water rose rapidly in
its pH when it was boiled.

In order to estimate the buffer action of the breathing water
titrations were made, adding an increasing quantity of $\frac{n}{100}$ HCl or
NaOH solution to 100 cc of tap water, sea water, and redistilled water.
The results thus obtained were compared with each other, and a
graph was compiled from the data presented in the Table 2. (Fig.
2).

As will be seen in Fig 2 the tap water has a marked buffer action
on the acid side, and changes its pH but little, by the addition of
acid, when compared with redistilled water. On the alkali side,
however, the buffer action is not so distinct as that of the acid side,
and the pH rises with the addition of NaOH. But in regard to the
redistilled water, the pH rise due to alkali addition is far more promi-
nent than in the case of tap water, suggesting a great superiority of
tap water in buffer action.

2) *Sea water.* The sea water used for the experiment was supplied
by a water main from a sea water reservoir. As the reservoir was
renewed 3 to 4 times a day, the water was kept almost in its natural
condition, showing no remarkable change in temperature nor in density.
Though the density of sea water was of course subjected to a seasonal
change, the mean value was 1.02429 (15℃). The pH of sea water
also changes with the season, ranging between 8.10 and 8.25. But it
remained almost always in the neighbourhood of pH 8.20 during the
present investigation.

The buffer action of the sea water was shown in Fig. 2, compared
with that of the tap water and redistilled water. As can readly be
seen from this figure the sea water shows a remarkable buffer action
in both the acid and alkali sides, not changing its pH by addition
of 0.1 cc of $\frac{n}{10}$ acid or alkali solution.

With a view to examining the buffer action of sea water to a
wider extent a further titration was made. According to the results

TABLE 2.

	Acid side					Alkali side			
Water sample (cc)	HCl added (cc)	pH of fresh water	pH of sea water	pH of re-distilled water	Water sample (cc)	NaOH added (cc)	pH of fresh water	pH of sea water	pH of re-distilled water
100	0.0	6.80	8.20	6.05	100	0.0	6.80	8.20	6.05
,,	0.1	6.70	8.20	5.80	,,	0.1	7.10	8.20	6.40
,,	0.2	6.60	8.18	5.55	,,	0.2	7.20	8.21	6.60
,,	0.3	6.50	8.17	5.35	,,	0.3	7.55	8.22	6.95
,,	0.4	6.40	8.16	5.20	,,	0.4	7.80	8.24	7 40
,,	0.5	6.30	8.14	5.00	,,	0.5	8.10	8.25	8 05
,,	0.7	6.20	8.08	4.60	,,	0.7	8.50	8.26	9.00
,,	1.0	6.00	8.00	4.10	,,	1.0	8.80	8.30	9.70
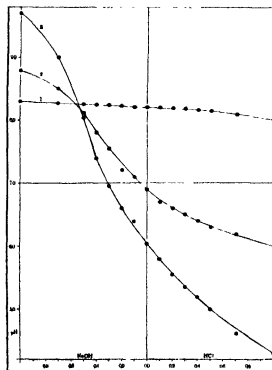
Fig. 2. Titration curves of breathing water compared with that of redistilled water.

Ordinate — quantity of 0.01 n solutions of HCl and NaOH in cc.

Abscissa — pH. (1 sea water 2 tap water. 3 redistilled water.)

listed in the following table an addition of 7 cc of $\frac{n}{100}$ HCl solution
changed the pH of sea water from 8.20 to 6.60, thus showing a
decrease in pH of 1.60; while addition of the same quantity of $\frac{n}{10}$
NaOH solution raised the pH from 8.20 to 8.85, showing an increase
in pH of only 0.65. Thus we notice that the change of pH due to

TABLE 3.

Acid side			Alkali side		
cc of water sample	cc of HCl $\left(\frac{n}{100}\right)$ added	pH of sea water	cc of water sample	cc of NaOH $\left(\frac{n}{100}\right)$ added	pH of sea water
100	0.0	8.20	100	0.0	8.20
,,	1.0	8.00	,,	1.0	8.30
,,	2.0	7.70	,,	2.0	8.45
,,	3.0	7.40	,,	3.0	8.60
,,	4.0	7.20	,,	4.0	8.65
,,	5.0	6.90	,,	5.0	8.70
,,	7.0	6.60	,,	7.0	8.85
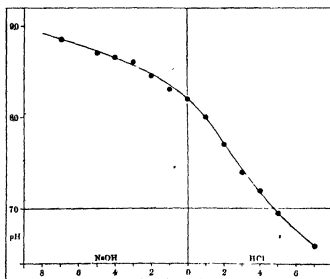
Fig. 3. Titration curve of sea water.
Ordinate — quantity of 0.01 n solutions of HCl and NaOH in cc.
Abscissa — pH.

the addition of the same quantity of the above solutions is by far
greater in the acid side than in the alkali side (Fig. 3).

VII. NATURE OF THE EXPERIMENT

The experiment was made 33 times in all during 1926 and 1927.
Among these 12 were Acidosis Experiments and 7 were Alkalosis
Experiments, all conducted on *Cyprinus*. Among the remaining 16
experiments 10 were Acidosis Experiments and 6 were Alkalosis
Experiments, all conducted with *Leuciscus*.

Of the 12 acidosis experiments made on *Cyprinus* the first 7 were
carried out with a view to induce acidosis by the low pH and the
O_2 deficiency in breathing water. But I was able to note by these
7 experiments that the lack of O_2 becomes a distinct cause of acidosis
while the low pH of water does not. Therefore in the next 2 experi-
ments the pH of the breathing water was not particulary lowered,
and only the effect of the O_2 deficiency was observed. From the
result of these 2 experiments it was ascertained that the O. deficiency
alone causes the acidosis, unassisted by the low pH of the water. In
Exp. 10 to 11, therefore, the O_2 content of the breathing water was
kept normal, and the effect of the low pH water was singly tested,
thus affirming that the low pH of water never produces the acidosis
in *Cyprinus*.

Among the 7 alkalosis experiments the first 2 showed that the
high pH of breathing water produces the alkalosis by 15 hours later,
and the O_2 deficiency of water in these experiments seemed to have
nothing to do with the alkalosis. The 3rd experiment was carried
out with the hope of inducing the alkalosis more rapidly than in the
former case, by raising the water pH. As the aim of the 3rd
experiment was fairly well attained, I verified these results positively
by Exp. 4 and 5.

In the acidosis experiment on *Leuciscus* the first 6 experiments
(Exp. 1–6) were made in order to study whether in *Leuciscus* also,
acidosis is brought about by the O_2 deficit. This relation being fully
determined by these experiments, the next three (Exp. 7–9) were
undertaken to test if the acidosis may be produced by the law pH
of the water. As these 3 experiments showed the result that this

fish displayed an apparent acidosis due to the low pH of water, a further experiment (Exp 10) was conducted in order to test the recovery of the acidosis.

With regard to the alkalosis of *Leuciscus*, 6 experiments were made. Of these experiments the first 2 were not entirely successful on account of the O_2 deficiency (Exp. 1) and the excessive high pH of the breathing water, resulting in no alkalosis in spite of the high pH of breathing water. Whereas in the 3rd experiment, in which the water was frequently renewed, the alkalosis was fairly well brought about. Hereupon I conducted 2 further experiments (Exp. 4-5) to affirm the results of the 3rd experiment. As the preceding 3 experiments demonstrated that the alkalosis of *Leuciscus* may readily be produced by the high pH of water, the 6th experiment was planned. In the 6th experiment the alkalosis and acidosis were alternately caused, thus demonstrating the possibility of successive changes of these conditions in one and the same fish.

CHAPTER II.

GENERAL PART 2. RESULTS.

1 THE CHANGE OF THE pH OF BREATHING WATER

In the experiment on alkalosis in which the initial pH of breathing water was considerably raised, the pH value decreased with time because of the respiration of the fishes. In the acidosis experiment in which the initial pH of breathing water was considerably depressed this relation was quite reversed, increasing the pH value in the course of the experiment. Such change of the pH was, however, by far more conspicuous in the alkalosis experiment than in the acidosis experiment. In other words, the decrease of the high pH due to the respiration of fishes was far more marked than the increase of pH due to the same cause. The cause of the decrease of high pH of water must be mainly due to the neutralization of alkali by the carbonic acid evolved by the respiration of fishes. While the rise of the low pH might partly be attributed to the neutralization of acidity by the alkali reserve of fish blood, it may partly be ascribed to the consumption of acid by the oxydizable matter excreted from

the fish body such as mucus and other excretions.

The rate of the pH change of breathing water, however, varies with the experiment, as it is related to the quantity and species of fishes, temperature, and the method of experiment.

Increase of the water pH in the experiment on Cyprinus (see Table 4). In the Acidosis Experiments 1 to 3 which were almost equal in experimental conditions, (fish about 456 gms. temp. 12°–15°) the initial pH of 3.70 to 3.90 rose to from 4.10 to 1.20 in the course of 7 hours, indicating an increase of pH 0.30.

In Exp. 4 and 5 (fish about 400 gms. temp 25°–26°) the pH rose 0.7 and 0.4 respectively, in the course of 3.5 hours, hinting that the rapid rise in these experiments may be due to the high temperature of breathing water.

Exp. 6 and 7 also showed a noticeable rise, but the cause of the rapid rise in these cases may be ascribed to both the high temperature and the larger number of the fishes kept in a jar.

The pH rise found in Exp 10 to Exp. 12 was most remarkable. For instance, in Exp. 11 the pH rose 2 55 within 3 hours, and in Exp. 12 increased 0.20 in the course of only 20 minutes. Such a distinct increase of pH as seen in these experiments may be referred to the escape of CO_2 from water by the aeration made with the object of preventing the O_2 deficiency.

TABLE 4.

I *Cyprinus* (shows increase of water pH)

(Exp. 7 and 8 were curtailed here as acid was not used in these 2 Exps)

No of Exp	pH		Duration of respiration	Water temp.	Fish	
	Range of Change	Total Change			No	Total weight (gms)
Acid Exp. 1	3.90–4.20	0.30	7 hrs	13.5'–15.2°	1	470
,, ,, 2	3.80–4.10	0.30	7 ,,	12.0°–12.2°	1	464
,, ,, 3	3.70–4.12	0.42	7 ,,	10.0°–12.0°	1	470
,, ,, 4	3.90–4.60	0.70	5 ,,	25.0°–26.3°	1	380
,, ,, 5	3.80–4.20	0.40	3.5 ,,	25.0°–26.2°	1	415
,, ,, 6	3.90–4.50	0.60	5 ,,	20.0°–20.5°	6	2418
,, ,, 9	6.70–5.60	1.10	3 ,,	4.0°–15.8°	1	780
,, ,, 10	3.70–4.40	0.70	3 ,,	8.7°–20.5°	6	3421
,, ,, 11	3.60–6.15	2.55'	3 ,,	8.0°–15.0°	6	3423
,, ,, 12	3.70–3.90	0.20	20 mns	3.5°–17.0°	2	1158

Decrease of the water pH in the experiment of Cyprinus. As above
mentioned the high pH of the breathing water was rapidly lowered by
the respiration of *Cyprinus*. But, as the experiment on Alkalosis
was carried out adding the alkali constantly, the pH showed no regular
change. And hence it is impossible to observe the mode of the pH
decrease by the results of these experiments Therefore I conducted
another experiment in order to make this relation clear.

Experiment 1.

In this experiment the decrease of the natural tap water due to
respiration was examined. The water showed the initial pH to be
7.45 and the temperature to be about 12°C. Two determinations
were made, using 20 liters of water without renewing until 1.5 hours
later.

The dimentions of fishes used were as follows.

	Body length (cm)	Body height (cm)	Body weight (gms)
Case 1	30.5	8.0	420
Case 2	31.0	7.9	397

TABLE 5.

Time in minutes	Case I			Case II		
	pH	Water temp	Resp freq (per minute)	pH	Water temp.	Resp freq (per minute)
0	7.45	12.0°	--	7.45	12.2°	39
10	7.10	,,	29	7.00	,,	--
20	6.95	,	--	6.90	,,	40
30	6.86	,,	26	6.85	,,	--
40	6.82	,,	--	6.80	,,	38
60	6.80	,,	19	6.80	,,	--
80	6.80	,,	--	6.70	,,	38

The above results show that the respiration of a *Cyprinus* lowers
the pH of breathing water by pH 0.70 in 1.5 hours (Fig. 4).
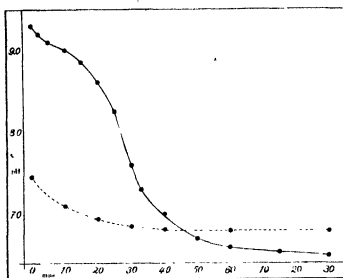
Fig 4 Decrease of pH in breathing water due to respiration of fish
Ordinate — pH. Abscissa — time in minutes
Continued line — decrease of pH which was raised by addding alkali
previously.
Broken line — decrease of pH in tap water

Experiment 2

The pH of breathing water used for the present experiment was
raised to 9.30 by adding about 22 cc. of $\frac{n}{5}$ NaOH solution to 20
liters of water. The temperature was kept 14.3°–15.0°C, and the
water was not renewed until the experiment was finished. 3 fishes
were used, weighing 1425 gms in total weight. The dimentions of
each fish were as follows.

	Body length (cm)	Body height (cm)	Body weight (gms)
1.	30.9	8.8	475
2.	30.3	8.1	430
3.	31.0	8.9	420

As can be seen in the Table 6. the pH of water began to
decrease immediately after the introduction of fishes, and the initial

TABLE 6.

Time (minutes)	pH	Water temp.	Resp. freq.
0	9.30	14.3	48
2	9.20	14.3	-
5	9.10	14.3	-
10	9.00	14.3	46
15	8.85	14.3	-
20	8.60	14.3	-
25	8.25	14.4	51
30	7.60	14.5	-
40	7.00	14.7	-
50	6.70	15.0	55
60	6.60	15.0	-
1ʰ—15	6.55	15.0	-
1—30	6.50	15.0	50

pH 9.30 fell to 7.60 after 30 minutes. 1 hour later the pH became 6.60 and at last reached the lowest value of 6.50 in 1.5 hours. The rate of decreasing was observed to be most rapid from the 20th to 30th minutes, but became very slow from the 50th minute on (Fig. 4).

In short, the experiments shows us that the pH of breathing water decreases at a rapid rate due to the respiration of fishes. The lowest values reached in the above 2 experiments ranged from 6.80 to 6.50. But the lowest value attained in the experiment on *Leuciscus* (Acidosis Exp. 5, 6) was pH 5.80 (Table 7). The lowest pH attainable may be still further lowered by lengthening the duration and by increasing the number of fish.

But we may conclude that the lowest pH attainable by the respiration of fishes in a relatively short time is in the neighbourhood of pH 6.00. Though this pH is very low in comparison to the ordinary pH of tap water, yet it is much higher than the acidity of tap water saturated with carbonic acid (pH 3.70).

Increase of the water pH in the Experiment on Leuciscus. The increase of the pH in the Acidosis experiment was observed to be very marked. In Exp. 7 in which 3 fishes were used, the initial pH 3.04 rose to 3.57 in the course of 2 hours (water temp. 11.0°–12.2°C).

In Exp. 8, in which 3 fishes were used and which was made under a little higher temperature, the rate of pH rise was more prominent than in the former experiment, showing a rise of pH 0.27 within 2 hours. Exp. 9 (one fish) also showed a marked rise, giving an increase

of pH 0.10 in 40 minutes.

Decrease of the water pH in the Experiment on Leuciscus. The decrease of the pH in the experiment on *Leuciscus* was also markedly observed. In the Acidosis Exp. 1 in which 3 fishes were employed (1456 gms in total weight, water temp. 10.4°–12.0°C) the initial pH 8.15 fell to 7.00 after respiration for 5 hours. As the Acidosis Exp. 2 was carried out using 5 fishes under a higher temperature, the decrease of the pH was very distinct, showing a fall of pH 1.16 within 2.5 hours. The Acidosis Exp. 3 and 4 in which 3 fishes in each were used also resulted in similar changes. Though the above 4 experiments were made using sea water, the following 2 experiments in which fresh water was used showed similar results. In the Acidosis Exp. 5 which was conducted using 3 fishes the initial pH 7.50 fell to 5.80 in the course of 2 hours, and the Acidosis Exp. 6 also showed a similar result, in the main.

The pH change of the breathing water due to the respiration of *Leuciscus* may be tabulated as follows.

TABLE 7.

No. of Exp.	pH		Duration of respiration	Water temp.	Fish	
	Range of Change	Total Change			Number	Total weight (gms)
			(Decrease)			
Acid. Exp. 1	8.15–7.00	1.15	5.ʰ 00ᵐ	10.4°–12.0°	3	1456
,, ,, 2	8.16–7.00	1.16	2. 30	11.0°–12.2°	5	287
,, ,, 3	8.20–7.10	1.10	2. 00	10.5°–10.8°	3	2024
,, ,, 4	8.18–7.10	1.08	2. 30	10.8°–11.5°	3	1873
,, ,, 5	7.50–5.80	1.70	4. 00	14.8°–18.0°	3	1479
,, ,, 6	7.40–5.80	1.60	2. 30	12.2°–13.5°	4	1929
			(Increase)			
,, ,, 7	3.40–3.57	0.17	2. 00	11.0°–12.2°	3	1565
,, ,, 8	3.40–3.67	0.27	2. 00	12.7°–13.7°	3	1482
,, ,, 9 A	3.40–3.50	0.10	0. 40	12.0°–12.1°	1	451
,, ,, 9 B	3.40–3.50	0.10	0. 40	11.9°–12.1°	1	417

II. THE CHANGE OF O₂ CONTENT IN THE BREATHING WATER

The O₂ content of breathing water. The O₂ content of the breathing water varied not only with the temperature but also changed

according to whether it was fresh or sea water. But, the O_2 content
of the water used in the present work never reached saturation at
any temperature. Consequently, the fresh and sea water showed
some (0.87 cc in fresh water and 0.59 cc in sea water in mean, per
liter) unsaturated portion (difference between theoretical value and
observed content). The O_2 content observed will be seen in the
following table.

TABLE 8.

	No of Exp.	Fresh water				No of Exp	Sea water			
		Water temp	O_2 quantity (cc per l)		Unsaturated portion		Water temp	O_2 quantity (cc per l)		Unsaturated portion
			Saturated	Observed				Saturated	Observed	
Ardosis	1	17.0	6.75	6.22	0.53	1	10.4	6.90	6.13	0.77
	2	12.0	7.52	6.06	1.46	2	11.0	6.80	6.17	0.63
	3	11.4	7.63	6.53	1.10	3	10.5	6.87	6.64	0.23
	4	25.0	5.78	6.36	−0.58	4	10.3	6.92	6.74	0.18
	5	10.3	7.80	6.54	1.26	7	12.6	6.59	5.69	0.60
	6	6.3	8.68	6.99	1.69	8	12.7	6.57	5.96	0.61
	7	4.5	9.00	8.56	0.44	9 A	12.0	6.53	6.67	−0.14
	8	4.1	9.07	8.10	0.97	9 B	11.9	6.54	6.00	0.54
	9	4.0	9.14	7.88	1.26	10	11.6	6.70	5.92	0.78
	10	8.7	8.10	7.65	0.45					
	11	5.0	8.91	8.19	0.72	1	11.9	6.54	5.99	0 55
	12	3.5	9.20	8.12	1.08	3	13.2	6.50	5.70	0.80
Alkalosis	1	8.3	8.20	7.38	0.82	4	13.7	6.49	6.70	−0.21
	2	13.2	7.30	7.02	0.28	5	13.3	6.43	6.49	1.21
	3	7.6	8.34	7.97	0.37	6	13.6	6.45	5.68	0.77
	4	7.0	8.47	7.92	0 55					
	5	5.0	8.91	7.91	1 00					
	Mean	8.99	8 16	7.40	0.87	Mean	12 0	6.64	6.09	0.59

The change of O_2 content. For the determination of respiratory
exchange the O_2 content of breathing water has to be estimated,
allowing the fishes to respire in a closed vessel. But in my present
work there was no need of such a particular method, for my purpose
was only to determine the relative change of the O_2 content in the
course of each experiment. Therefore the O_2 content was determined
by WINKLER's method on the samples taken at definite intervals from
an open vessel in which the fishes were kept.

As can be seen in the following table, the results thus obtained
show that the O_2 content of the breathing water rapidly decreases
with time.

TABLE 9 (a).

(Decrease of O₂ content of water by the respiration of *Cyprinus*).

TABLE 9 (b).

(Decrease of O₂ content of water by the respiration of *Leuciscus*).

The rate of the O_2 consumption in these cases, of course, depends on the water temperature, and the quantity and size of the fishes. In the Acidosis Experiments on *Cyprinus* (Alkalosis experiments were disregarded as the water was renewed at intervals) the O_2 decrease was most rapid during the first 1 hour, especially in the first 30 minutes. After that, however, the decrease became very slow. Among the results listed in the Table 9 (a) the most rapid decrease was seen in Exp. 9 in which 5.9 cc of O_2 was consumed in the first 30 minutes. In Exp. 6, Exp. 8 and Exp. 10, about 5.3, 4.3, 4.2, and 4.0 cc of O_2 decreased respectively in the first 30 minutes, while in the other experiment the O_2 decrease was slower than in the above cases.
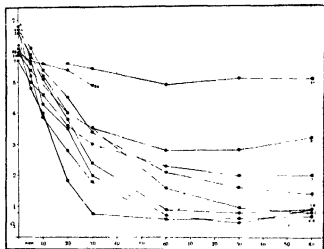
Fig. 5   Decrease of O_2 content in breathing water due to respiration of fishes (*Leuciscus*, Acidosis, Exp 1–10). Numerals attached to curves denote the No of experiment
Ordinate — O_2 quantity in cc, Abscissa — time in minutes

In the Acidosis Exp. on *Leuciscus* the O_2 decreased also rapidly in the first 30 minutes and attained almost the minimum within 1 hour, showing no marked change after that. The most rapid decrease was seen in Exp. 2 in which 5.4 cc of O_2 was consumed in the first 30 minutes. In Exp. 4, Exp. 3, Exp. 6 and Exp. 5 about 4.9, 4.8, 4.2 and 3.3 cc of O_2 decreased respectively in the first 30 minutes.

In the other experiments the O_2 decrease was slower than those in the above cases (Fig. 5).

As to why the rate of O_2 decrease varied so with the experiment, the readers are referred to the experimental condition stated in the description of each experiment.

Among the Acidosis Exp. of *Leuciscus*, Exp. 7, 8 and 9 showed a relatively slow O_2 decrease in spite of the fact that the quantity of fish was never less and the water temperature was never lower than those of the other experiments. On the other hand, the Alkalosis Exp. 1 showed a rapid decrease of O_2. And hence, this is likely to prove that the O_2 is more readily absorbed by fishes in the alkali water than in the acid water.

PACKARD (52) reported that the resistance of *Fundlus* to the lack of O_2 may be increased by raising the blood pH by the injection of $NaHCO_3$, and may be decreased by lowering the blood pH by the injection of acetic acid. The question of resistance in this case may be accounted for by the theory that an increase or decrease in the alkalinity or acidity of the plasma favours or retards the oxydation phenomena.

In my 3 experiments above mentioned (*Leuciscus*, Acid. Exp. 7, 8, 9) the blood pH was already lowered in the first 30 minutes. Consequently the oxydation of blood corpuscles might have been retarded by that time, thus decreasing the rate of O_2 consumption of fishes. In Alkalosis Exp. 1, on the contrary, the blood pH was raised in 30 minutes, so that the oxydation of blood corpuscle was accelerated, and thus increased the rate of O_2 consumption. The reason why such phenomena were not observed in the experiment of *Cyprinus* may be ascribed to the fact that *Cyprinus* needed a comparatively long time (over 1 hour) to change the blood pH, and therefore the effect of the altered pH on the O_2 consumption was not observed in the early part of the experiment, as will be seen from the change of pH of blood.

III THE CHANGE OF THE pH OF BLOOD

(A) THE pH OF THE BLOOD

1) *Cyprinus.* According to my former investigation, the normal pH of the blood of *Cyprinus* was found to be 7.60 in the mean of 31 determinations, ranging from pH 7.35 to 7.75 However 33 determinations made in the present investigation showed it to be pH 7.75 in mean, ranging between pH 7.20 and 8.20, thus showing a greater fluctuation than that of the former investigation. Summing up the above 2 investigations just stated the normal blood pH of *Cyprinus* is to be taken as 7.68, for the mean of the above two means.

The normal blood pH of *Cyprinus* found in the present investigation can be listed as follows.

Acidosis Exp.	No. 1.	7.62				
	,, 2.	7.70	Oct. 23–28 (1926)			
	,, 3.	7.75	Mean pH			
	,, 4.	7.75	7.72			
	,, 5.	7.80				
	,, 6.	7.60				
	No. 7.	7.79				
	,,	7.35				
	,,	7.20				
	,,	7.70				
	,,	7.85	Dec. 18–31 (1926)			
	No 8.	7.50	Mean pH			
	,,	7.87	7.71			
	,,	7.70				
	,,	7.85				
	,,	7.70				
	,,	7.83				
	,,	7.50				

Acidosis Exp.	No. 10.	7.50	
	No 11	7.75	
	,,	7.85	Jan. 1–17 (1927)
	,,	7.91	Mean pH
	No. 12	7.70	7.72
	,,	7.70	

Alkalosis Exp.	No 1	7.64	
	No. 2.	7.98	
	No. 3	7.97	March.
	,,	8.00	19–23 (1927)
	,,	8.20	Mean pH
	No 4.	7.95	7.93
	,,	7.57	
	No. 5.	7.85	
	,,	7.95	

Mean of all (33) determinations pH 7.75

As is seen in the above table some fishes showed so high a value that I first doubted the results, but it was confirmed to be certain by examining the other fishes of the same lots which were not used for the present experiment.

2) *Leuciscus.* The normal blood of the *Leuciscus* was also studied

in my previous paper, reporting the mean value of 5 determinations to be pH 7.44, ranging from 7.20 to 7.70. In the present investigation, however, the mean pH of 50 determinations was shown to be pH 7.17, ranging between pH 7.00 and 7.65. Averaging the results of the present and previous investigations the pH of the blood of *Leuciscus* may be taken as pH 7.42.

The normal pH of the blood of *Leuciscus* determined by the present investigation can be listed as follows.

Acidosis Exp. (Mean value pH 7.29, May 9-15.)		Acidosis Exp. (Mean pH 7.14, May 9-15.)		Alkalosis Exp (Mean pH 7.12, June 1-4.)	
No. 1.	7.50	No. 5.	7.06	No. 1.	7.03
No. 2.	7.35	,,	7.13	,,	7.30
,,	7.20	,,	7.33	,,	7.05
,,	7.25	No. 6.	7.07	No. 2	7.02
,,	7.45	,,	7.02	,,	7.01
,,	7.30	,,	7.03	,,	7.07
No. 3.	7.28	No. 7.	7.22	No. 3.	7.05
,,	7.07	,,	7.10	,,	7 65
,,	7.40	,,	7.22	,,	7.30
No. 4.	7.30	No. 8.	7.25	No. 4.	7.00
,,	7.18	,,	7.00	,,	7.01
,,	7.20	,,	7.27	,,	7.00
		No. 9.	7.15	No. 5.	7.20
		,,	7.20	,,	7.20
		No. 10.	7 03	,,	2.27
		,,	7.01	No. 6.	7.03
		,,	7.05	,,	7.32
		,,	7.07	,,	7.05

(B) THE CHANGE OF THE pH OF BLOOD

1) Acidosis.

1) *Acidosis in Cyprinus*. (Table 21-32, Fig. 6). 12 experiments which were made with a view to study the acidosis of *Cyprinus* can be classified into the following 5 categories according to their mode of the change of blood pH. .

1. Exp. 1-3. In these experiments the blood pH decreased with time, reaching the minimum after 6 hours. Thereafter, however, the

blood pH tended to increase inasmuch as the fresh tap water was supplied. After 24 hours the blood pH increased, reactionary to the former decrease, and exceeded the initial value, though, after this, it began to decrease and approximated the normal value by 48 hours afterwards. From the result of Exp. 2 it can be presumed that the pH decrease in Exp. 1 and 3 also might have occurred from one hour afterwards.

2. Exp. 4-5. The decrease of the blood pH in these 2 experiments was very rapid in comparison with the former 3 experiments, showing an outstanding decrease within 1 hour. The cause of such a rapid decrease may be ascribed to the high temperature of the breathing water.

3. Exp 6-7. These 2 experiments also showed a very rapid decrease of the blood pH. In Exp. 6 the blood pH commenced to decrease as soon as 30 minutes afterwards, attaining the minimum value by 2 hours later. Exp. 5 also showed a remarkable decrease in the mean value of 6 individuals The rapid decrease observed in these 2 experiments must be due to the sudden fall of O_2 tension in the breathing water.

4. Exp. 8-9. These two experiments were carried out keeping the water pH normal, but rapidly decreasing the O_2 of breathing water. The results showed that the blood pH distinctly decreased within 3 hours, thus showing the single effect of the O_2 deficiency in the breathing water. In Exp. 8 the fish were fed with tap water from 3 hours afterwards, so that the blood pH recovered its initial value, while in Exp. 9 the tap water was not supplied and consequently the fish maintained a low blood pH until the end of the experiment.

5. Exp. 10-12. These 3 experiments were made in order to test the single effect of the low pH of breathing water, eliminating the effect of the O_2 insufficiency. But in Exp. 10 the blood pH was lowered by the lack of O_2 in the water on account of the unsuccessful supply of O_2. In Exp. 11 any want of O_2 was avoided to the utmost. As a result, the blood pH never decreased in spite of the low pH of water. From 3 hours afterwards, however, the blood decreased markedly on account of a sudden decrease of O_2 after that time. Therefore our attention is attracted by the fact that the pH decrease which was observed in Exp. 1-7 was not due to the low pH of water,

but to the O_2 deficiency of water. In Exp. 12 also, the single effect
of low pH was examined, prolonging the duration of experiment, but
no effect of low pH was found. (In Fig. 6 the Exps. 6, 7, 8 and 10
were eliminated as the observed points shown in each series were
taken from the determinations made on different individuals).

Fig 6   Change of blood pH in the course of experiment (*Cyprinus*,
Acidosis Exp)
Ordinate   pH   Abscissa - time in hours.
Numerals attached to curves denote the No of experiment   Alphabet
signifies the individual of fish

2) *Acidosis in Leuciscus.* (Table 39- 49, Figs. 7–8). 10 experiments
which were conducted with *Leuciscus* can be classified into 3 groups
i. e., (1) Fall of pH due to O_2 want of water (Exp. 1–6) (2) Fall of
pH due to low pH of water (Exp. 7–9) (3) Recovery of the lowered
blood pH (Exp. 10).   Besides 3 fishes which were used in each
experiment 1 control fish which was kept in normal sea water was
employed in Exp. 2 and 6 for comparison.

1. Exp. 1–6. Among 6 experiments, Exp 1 commenced to de-
crease the blood pH by 1 hour later, which fell more and more
with the time. In Exp. 2 the pH fall which was caused within the
first 1 hour was examined, with the result that the blood pH once
rose within 10 minutes and began to fall from 20 to 30 minutes

afterwards. While the control fish showed but an indistinct change,
suggesting that the change found in the other fish is due to the
lack of O_2 in the water. Exp. 3 was a similar experiment to
formar one, and a similar result was observed except in one fish (C)
in which the blood pH began to decrease within 30 minutes, showing
no increase at the beginning. In Exp. 4 all fishes decreased the blood
pH with time, though one of them alone (A) once raised the pH by
30 minutes later. 2 fishes employed in Exp. 5 decreased the blood
pH after raising it within 30 to 60 minutes. In Exp. 6 the pH change
of all fishes well agreed, showing that it rises in 30 minutes, and
after this it decreases. In the control fish no change was observed
(Fig. 7).
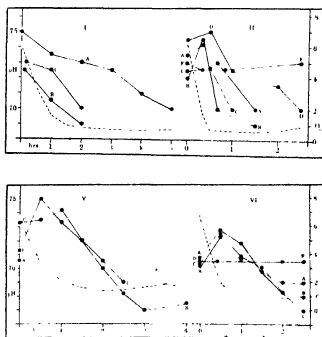
Fig. 7. Decrease of blood pH and O_2 content in breathing water
(*Leuciscus*, Acidosis Exp 1, 2, 5, 6)
Ordinate (left side) --- pH.
Ordinate (right side) --- O_2 in cc.
Abscissa -- time in hours

In short, the blood pH of *Leuciscus* decreases due to the O₂ want in breathing water. But 11 individuals among 14 fishes which were observed over 1 hour showed the apparent rise of pH by 30 minutes later, though the remaining 3 fishes showed some discrepancy. Therefore we can now conclude that in the acidosis caused by O₂ want blood pH decreases later after rising once by 30 minutes.

2. Exp. 7-9. The effect of the low pH of breathing water much differs from that of the O₂ deficiency. In Exps. 7 and 8 the blood pH showed a marked decrease within 30 minutes, and the decrease was most markedly observed in fishes which maintained the high initial pH. Notwithstanding such distinct decrease in experimental fish, control fish made almost no change, indicating that the pH change observed in the experimental fishes is due to the effect of the low pH of water. In Exp. 9 the observations were made 3 times within 30 minutes and the results showed that the pH decrease occurs as early as from 5 to 10 minutes later (Fig. 8)

Fig. 8. Decrease of blood pH in the course of experiment (*Leuciscus*, Acidosis Exp. 7-9).
Ordinate — pH. Abscissa — time in hours
Alphabet signifies the particular individual of fish

3. Exp. 10. In this experiment also the blood pH was rapidly decreased by the low pH of water, but it increased at a rapid rate when the fishes were replaced in the normal sea water, exceeding the initial pH from 40 minutes afterwards. Though this increase continued until 6 hours later, it began to decrease thereafter, approximating the normal pH by 48 hours afterwards.

To sum up, in *Leuciscus* the acidosis is caused either by lack of O_2 or by the low pH of the breathing water.

But the effect of these 2 causes differs in the point that the O_2 want raises the pH once before it decreases, while the low pH of water directly decreases the blood pH.

3) *Comparison of the acidosis of Cyprinus and Leuciscus.* A comparison of these two species can be designated as follows.

Difference
{
 I. *Leuciscus* induces acidosis by the low pH of breathing water, while *Cyprinus* does not.

 II. *Leuciscus* shows an apparent increase in blood pH prior to the subsequent decrease, while *Cyprinus* shows no such increase.
}

Similitude
{
 I. In both fishes the acidosis is caused by O_2 deficiency of water.

 II. Both fishes show reactionary increase of blood pH subsequent to the acidosis.
}

4) *Relation of acidosis to the O_2 lack of breathing water.* As it has been found that the O_2 want of breathing water becomes a main cause of the acidosis, a discussion will be given with regard to the relation which exists between acidosis and the O_2 want.

1. *Cyprinus.* In Exps. 1–5 the decrease of the O_2 content of water was relatively slow, and it increased rapidly from 7 hours afterwards because of the introduction of fresh tap water. In response to such O_2 change the blood pH reacted correspondingly, decreasing gradually by 7 hours later and increasing rapidly from 24 hours afterwards.

In Exp. 6 in which the O_2 content rapidly decreased within a few minutes, attaining about 24% of the initial content, the blood pH abruptly commenced to decrease from 30 minutes afterwards, and reached the minimum after 2 hours. Next, in Exp. 7 the O_2 content reached the minimum after 1 hour from the start, giving about 15% of the initial content. The blood pH change in accordance with this, decreasing rapidly from 1 hour afterwards, though it varied somewhat with the individual (Fig. 9).

Fig. 10 shows the decrease and increase of O_2 and blood pH in Exp. 8. This figure not only shows that the blood pH changes in association with the O_2 change but also that the degree of pH
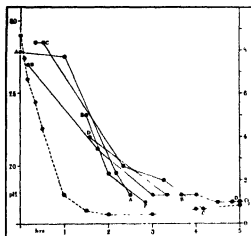
Fig 9. Relation of the lowering of blood pH to the decrease of
O_2 content of breathing water (*Cyprinus*, Acidosis Exp. 7).
Ordinate (left side) — pH
Ordinate (right side) — O_2 in cc
Abscissa — time in hours
Continued curve — lowering of blood pH
Broken curve — decrease of O_2.
Alphabet signifies the particular individual of fish

decrease in a definite time greatly differs with the individual.

In Exp. 9 the decrease of O_2 was so rapid that it reached the minimum (0.8% of initial content) after 1 hour. Accordingly the blood pH decreased very rapidly showing pH 6.80 after 3 hours. Exp. 10 also showed an intimate relation of pH to the O_2 decrease.

Exp. 11 and 12 were specially made to determine the relation of the pH to the O_2 change. In Exp. 11 the blood pH made no change until 3 hours later as the O_2 showed no remarkable decrease until that time. But as soon as O_2 began to decrease the blood pH also commenced to decrease. In Exp. 12 the blood pH was presumed to show no change until 4 hours later, inasmuch as the O_2 showed no change. After this, however, until 7 hours later the blood pH apparently decreased.

This was probably caused by the O_2 decrease observed from 4 hours afterwards.

From the results above mentioned we can designate the relation

of blood pH to the O$_2$ decrease as follows (5 experiments were cur-
tailed as they lacked observations until 1 hour later).
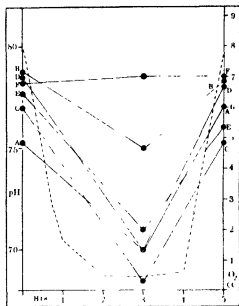
Fig. 10 Relation of the lowering of blood pH to the decrease of
O$_2$ content in breathing water *(Cyprinus, Acidosis Exp 8)*
Ordinate (left side)- pH
Ordinate (right side)-- O$_2$ in cc
Abscissa - time in hours
Alphabet signifies the particular individual of fish

TABLE 10.

No of Exp	Time required to induce the pH decrease	O$_2$ content when the pH began to decrease
Exp 2	1 h 00^m after the start	87% of initial content
,, 6	0, 50 ,,	99% ,, ,,
,, 7	0, 50 ,,	50% ,, ,,
,, 9	0, 10 ,,	24% ,, ,,
,, 10	0, 20 ,,	61% ,, ,,
,, 11	0,30-2 30 ,,	74% ,, ,,
,, 12	6,00 ,,	60% ,, ,,

The above table suggests to us that the greater the O$_2$ decrease
the shorter the time. For instance when the O$_2$ was left over 60%,

the blood pH did not begin to decrease. While it began decrease as early as from 10 to 30 minutes afterwards when the O_2 decreased to 22-50%. A discrepancy which was found in Exp. 10 may be due to the individuality of fish or some other experimental conditions.

The rate of the pH decrease was rapid in Exp. 6-10, moderate in Exp. 1-6, and slowest in Exp. 11-12. These facts also show that the rate of the decrease of blood pH is proportional to that of the O_2 decrease.

2. *Leuciscus.* In Exp. 1 the rate of O_2 decrease was very slow in comparison with the following experiments, and in accord with this, the acidosis was also very slow (Fig. 7). Alkalosis which might have occurred previously to the acidosis was not found on account of the lack of observation. In Exp. 2 in which the O_2 decreased very rapidly the blood pH showed a rise after 20 minutes, and after this it began to fall rapidly, giving a lower value than the initial pH. The control fish, however, showed almost no change, thus demonstrating that the pH change observed in this case is due to nothing but the effect of the O_2 lack in the water (Fig. 7).

With regard to Exp. 3, in which the O_2 reached the minimum after 1 hour, the change of blood pH varied with the individual. A fish induced alkalosis when the O_2 decreased to 36% and caused acidosis when the O_2 attained the minimum. While another fish evenly decreased the blood pH from beginning to end. In Exp. 4 the O_2 decrease was so rapid that it became 0.9% of the initial value by 1 hour later, but the alkalosis which preceded the acidosis was not observed. Although the O_2 decrease in Exp. 5 was slower than in the former experiment, the alkalosis appeared first and was followed by the acidosis (Fig. 7). In Exp. 6 the O_2 decrease was almost as rapid as in Exp. 4 and apparent alkalosis preceded the acidosis. While the control fish which felt no O_2 want, never changed the blood pH throughout the experiment, thus making certain that the pH decrease of blood undoubtedly is due to the O_2 want of water (Fig. 7).

In summing up the above descriptions, we are led to conclude that the acidosis which is caused by the O_2 deficiency of breathing water is usually preceded by a light alkalosis. This alkalosis occurs as soon as the O_2 begins to decrease, and reaches the maximum when the O_2 content becomes 14-15% of the initial value. The more rapid

the decrease the earlier the acidosis. Furthermore we notice the fact
that the typical change of the blood pH in which the acidosis is
preceded by an alkalosis appears to be caused by an extraordinarily
rapid decrease of O_2.

2) Alkalosis.

1) Alkalosis in Cyprinus (Table 34–38, Fig. 11). In the five
experiments of alkalosis the trends of the pH change varied more
or less with the experiment, although in general tendency they accorded
well.

Exp. 1 showed that the blood pH showed almost no change till
after 3 hours, but after this it gradually rose until it attained the
maximum (pH 8.00) after 15 hours. Thereafter however, the pH
gradually fell. Exp. 2 showed the peculiar tendency of the pH to
fall before it began to rise after 4 hours, and after this the pH rose,
exceeding 8.00 by 24 hours later. In Exp. 3 the change of pH was
not observed until 4 hours later, but after this time the pH obviously

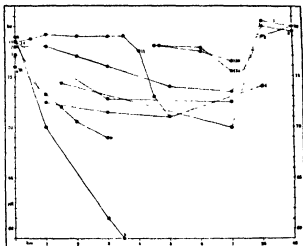Fig. 11. Increase of blood pH in the course of experiment (*Cyprinus*, Exp 1–5).

Ordinate — pH. Abscissa — time in hours
Numerals attached to curves denote the No. of experiment.
Alphabet signifies the particular individual of fish

rose, reaching the maximum pH of 8.35 by 24 hours afterwards. From this time on howeer, the pH showed a slight decrease, though it yet maintained a little higher value than the initial pH. The fact that the blood pH rose as distinctly as just mentioned, notwithstanding the O_2 decrease of water, emphasizes the effect of the high pH water on the blood pH. Exp. 4 showed also a like tendency to Exp. 3 in the main, giving the highest pH after five hours. Thereafter the blood pH tended to decrease slightly until 48 hours afterwards, though it was still higher than the initial pH. In this experiment also, the O_2 content of water decreased markedly, so that the rise of the blood pH must be due to the effect of the high pH of water which suppressed the effect of the O_2 deficiency of breathing water.

In Exp. 5 the blood pH showed a decrease at first and attained the minimum after 30 minutes, but after this it rose exceedingly, and reached the highest value 5 hours later. Thereafter the pH slowly fell until 72 hours later, though it was yet slightly higher than the initial value.

Looking through the above results, one will be struck by the fact that in Exp. 1 and 5 the fishes show distinct acidosis before they show the alkalosis. Of the other 3 experiments Exp. 3 and 4 lacked observation until after 30 minutes. Therefore it has not been known whether these experiments also brought about acidosis or not. But the fact the remaining one experiment (Exp. 1) also showed an acidosis, though only slightly, we may be able to surmise that Exp. 3 and 4 also might have caused the acidosis. As regards the cause of this acidosis it may probably be attributed to the decrease of O_2 in breathing water. In short, I am inclined to conclude that in *Cyprinus* the alkalosis is caused by the high pH of breathing water, although this alkalosis is, in the above cases, preceded by an acidosis due to O_2 want of water.

2) *Alkalosis in Leuciscus.* (Tables 50–55, Figs. 12–13). Among the 6 experiments the first 2 were not successful, but the others brought about remarkable alkalosis by the high pH of water.

Though Exp. 1 and 2 showed the alkalosis by 30 minutes later, it suddenly turned into the acidosis. In Exp. 3 the alkalosis began with the start of the experiment and the blood pH reached the maximum after 1.5 to 2 hours. But from 2 hours afterwards the

blood pH began to decrease, and gradually approximated the normal value by 48 hours later. This experiment is somewhat allied to the alkalosis experiment on *Cyprinus*, in that the pH rose rapidly at the beginning and continued for a long while.

   As the former experiment showed that the blood pH of *Leuciscus* markedly rises within 1 hour, Exp. 4 was carried out with a view to determine the mode of pH rise within 1 hour. The result showed that the blood pH rises rapidly immediately after the fishes are put in the high pH water giving an extraordinarily high blood pH after 2 hours.

   One fish alone, which was highly weakened, lowered the pH 2 hours later. It was a remarkable matter in this experiment that the acidosis which was observed in the alkalosis experiment of *Cyprinus* was not found.

   Exp. 5 showed a similar result to that of Exp. 3, raising the blood pH rapidly with the start of the experiment. The blood pH thus reached the highest value by 1 to 2 hours later. From 2 hours afterwards, however, the blood pH tended to decrease, notwithstanding that it still remained in the high pH water. Even after 24 hours the blood pH kept a much higher value then the initial pH (Fig. 12).
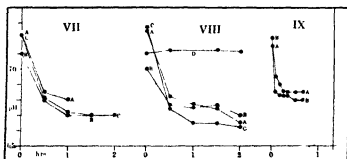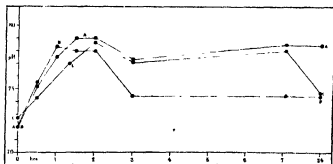

   Fig 12   Change of blood pH in the course of experiment (*Leuciscus*, Alkalosis Exp. 5).
   Ordinate — pH.   Abscissa — time in hours
   Alphabet signifies the particular individual of fish

   In Exp. 6 the acidosis and the alkalosis were alternately and successively caused, in order to demonstrate accurately that the acidosis

and the alkalosis can be induced by forcing the fishes to respire in the acidified or the alkalified water.

In this experiment the alkalosis commenced with the outset of the experiment, giving the highest blood pH after 1.5 hours. As the fishes were transferred to the low pH water after this, their pH soon began to fall very rapidly. And the high pH which was attained by the alkalosis of 1.5 hours fell rapidly below the initial value within 10 minutes. Thereafter the fall of the blood pH continued until it reached the lowest value of pH 6.75 after 30 minutes. At this time i. e. 2 hours and 10 minutes after the experiment started, the fishes were replaced in the high pH water, with the result that the blood pH which was depressed by the preceding acidosis soon began to rise, and after 40 minutes it by far exceeded the initial value (Fig. 13).
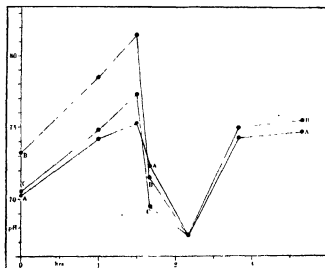
Fig. 13. Change of blood pH in the course of experiment (*Leucis-cus*, Alkalosis Exp. 6).
    Ordinate — pH. Abscissa — time in hours.
    Alphabet signifies the particular individual of fish.

Among the experiments above mentioned Exp. 1 and 2, which showed no typical alkalosis, may be regarded as being affected by the O_2 want of water (Exp. 1) and the weakness of the fishes (Exp.

2), as either of these two conditions may become a direct cause of acidosis. In short, it deserves a special mention that the alkalosis in *Leuciscus* which is caused by the high pH of water is not preceded by the acidosis.

   3) *Comparison of the alkalosis of Cyprinus and Leuciscus.* As before mentioned, both *Cyprinus* and *Leuciscus* induce the alkalosis by the high pH of water, but as regards the detailed mode of alkalosis, they differ from each other. In the first place, in *Leuciscus* the alkalosis is caused very rapidly, within 1 to 2 hours. While in *Cyprinus* it needed as long as from 4 to 24 hours. In the second, *Cyprinus* showed slight acidosis before inducing an apparent alkalosis, but *Leuciscus* directly showed the alkalosis. Thirdly, the highest blood pH (8.35) attainable by *Cyprinus* seems to be higher than that (8.15) attainable by *Leuciscus*

(C) CONCLUSION

I) Acidosis in *Cyprinus.*

   1) In *Cyprinus* the acidosis is caused by the O_2 decrease of the breathing water (Exp. 1-7).

   2) The rate of the pH decrease in the above case is proportional to the rate of the O_2 decrease. This can be verified by comparing the results of Exp. 1-4 with those of Exp. 6-9.

   3) The lowest limit of the blood pH lowered by the O_2 want of the breathing water seems to be in the neighbourhood of pH 6.80 as long as fishes survive (Exp. 9-10).

   4) The fishes in which the acidosis was caused by the O_2 want of breathing water raise the blood pH extraordinarily, when they are replaced in natural tap water, much exceeding the initial value (Exp. 1-4).

II) Acidosis in *Leuciscus.*

   1) In *Leuciscus* the acidosis is caused by the O_2 decrease of the breathing water (Exp. 1-6).

   2) The acidosis of *Leuciscus* caused by the O_2 want of breathing water is preceded by a small but positive alkalosis which occurs within 30 minutes (Exp. 1-6).

   3) The lowest limit of the blood pH lowered by the O_2 want of

the breathing water seems to be in the neighbourhood of pH 6.70, so long as the fishes survive (Exp. 10).

4) *Leuciscus* decreases its blood pH rapidly by breathing the low pH (3.40) water (Exp. 7-10).

5) The acidosis which is caused by breathing the low pH water can readily be recovered by making the fishes breath natural tap water. In this case the blood pH rises within a few hours, exceeding the initial value, and thereafter it approximates the normal value within 48 hours (Exp. 10)

III) Alkalosis in *Cyprinus.*

1) In *Cyprinus* the alkalosis is caused by breathing the high pH (9.00+) water (Exp. 1-5).

2) In this alkalosis the blood pH rises after once showing a small acidosis.

3) The acidosis occurring in this case seems to be due to the O_2 want of water (Exp. 1-2).

4) As far as the present data are concerned, the rise of the blood pH due to high pH of water reaches the maximum within about 5 hours.

5) The blood pH rose as high as pH 8.35 in alkalosis, yet the fishes recovered their activity.

IV) Alkalosis in *Leuciscus*

1) In *Leuciscus* also the alkalosis is caused by breathing the high pH (9.00+) water.

2) In the alkalosis of *Leuciscus* the blood pH begins to rise with the start of the experiment, and is never preceded by any acidosis.

3) The highest blood pH attained in the alkalosis was 8.15, yet the fish recovered normal activity.

4) In *Leuciscus* the acidosis and alkalosis can be alternately and rapidly caused without any indication of injury to the fish.

5) In *Leuciscus* both the acidosis and alkalosis are far more readily producible than in *Cyprinus.* Indeed the acidosis such as that due to low pH water (3.40) is almost instantaneously brought about, though the alkalosis is not so rapid as acidosis.

V) The chief difference between the present two species regarding the acidosis consists in the point that in *Leuciscus* the acidosis is

caused very readily by the high pH of breathing water, while in *Cyprinus* it is never brought about by the same cause.

IV. THE CHANGE OF THE CO_2-CONTENT OF BLOOD

(A) THE CO_2-CONTENT OF THE BLOOD.

1) *Cyprinus*. It was pointed out in my previous investigation (44) that the CO_2-content of the normal blood of *Cyprinus* was found to be 45.2 vol% in mean (from 16 individuals), ranging from 28.3 to 58.7 vol%.

The results of the present investigation, which were determined on 26 individuals, showed this to be 44.6 vol% in mean, ranging between 33.4 and 68.4 vol%. Consequently the mean of the two investigations (42 individuals) became 44.83 vol%, showing a very close approximation to the results of WASTLE (44), who gave 44.86 vol%.

2) *Leuciscus*. With regard to the CO_2-content of the normal blood of *Leuciscus* I reported its mean value to be 18.3 vol% (from 5 individuals) in my previous paper (26), ranging from 16.4 to 20.2 vol%. In the present investigation, in which 48 individuals were examined, this value was found to be 16.5 vol% in mean, and ranged between 10.8 and 23.7 vol%. Therefore the mean of the two investigations (53 individuals) gives the normal content to be 16.67 vol%.

(B) THE CHANGE OF THE CO_2 CONTENT OF BLOOD

I) Acidosis.

1) *Acidosis in Cyprinus*. (Tables 21–33, Fig. 14). The change of the CO_2-content of blood shows a close resemblance to that of the blood pH, as can be noticed by comparing Fig 14 with Fig. 6.

In Exp. 1–3 the CO_2-content showed a minimum value within 6 hours. After this it tended to increase and exceeded the normal value after 24 hours, as the fishes were supplied with tap water. Though the CO_2-content thus changed with the pH in the main, the pH curve sometimes conflicted with the curve of CO_2-content because of the fact that even for a definite pH the CO_2-content varies to a considerable extent. In Exp. 4–5 also the CO_2-content changed with the pH. In Exp. 6, in which 6 individuals were employed, the curve of CO_2-content

showed some irregularity on account of the different individuals used for each observed points, and hence the curve was curtailed from Fig. 14. In Exp. 7 the CO$_2$-content decreased far more rapidly than the pH, owing to a low value of CO$_2$-content for a definite pH.

In Exp. 8 in which the pH showed a regular change, the CO$_2$-content changed a little irregularly. For instance, some individuals of this experiment increased in CO$_2$-content with the recovery of pH, while in other individuals the CO$_2$-content continued to decrease, notwithstanding the pH increases. The change of the CO$_2$-content in Exp. 9 showed a good agreement with that of the pH. Exp. 10 showed an irregular change like Exp. 6, as each determination in this series was made on a different individual. Accordingly the curve was not given in Fig. 14. In Exp. 11 also, the CO$_2$-content changed somewhat irregularly, though in general trend it decreased with time. In one of the fishes, designated A, on which 9 determinations were made, the CO$_2$-content changed very irregularly until it began to decrease regularly from 3,5 hours afterwards. In Exp. 12 the change of the blood pH was first markedly recognized from 7 hours afterwards, while the CO$_2$-content notably changed till that time (Fig. 14).
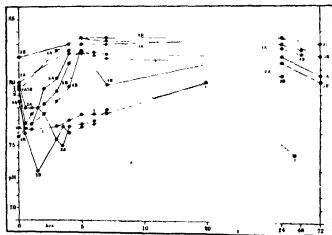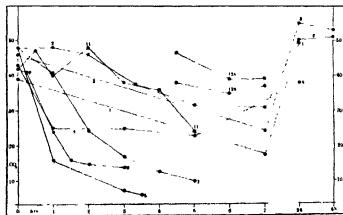

Fig. 14. Change of CO$_2$-content in blood in the course of experiment (*Cyprinus*, Acidosis Exp. 1-12).
    Ordinate — pH. Abscissa — time in hours.
    Numerals attached to curves denote the No. of experiment
    Alphabet signifies the particular individual of fish.

In short, the change of the CO_2-content in the acidosis of *Cyprinus* does not show close parallelism to the change of pH, although in general trend the CO_2-content also decreases with the time.

2) *Acidosis in Leuciscus.* The change of the CO_2-content in the acidosis of *Leuciscus* appeared to vary with the cause of acidosis. In Exp. 1 the CO_2-content changed in accordance with the change of the blood pH, decreasing with time. In Exp. 2, in which the pH rose a little within 30 minutes, the change of the CO_2-content agreed with the pH change in some fishes but not others. While the control fish showed only a little change, revealing that the change in CO_2 content in other fishes doubtlessly was due to the want of O_2 in the breathing water.

In Exp. 3 in which the pH fell gradually with time, the CO_2-content decreased in association with the pH change. In Exp. 4 also the CO_2-content decreased with the pH

In Exp. 5 and 6 the CO_2-content changed in a similar manner. In both of them the CO_2-content made a maximum rise after 30 minutes, and after this it tended to decrease, reaching the minimum value after 2 to 25 hours. Thus these two experiments showed parallelism in the change of CO_2-content and pH (Fig. 15).
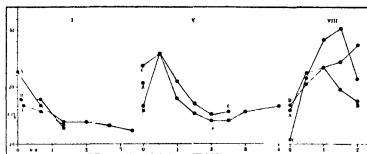
Fig. 15. Change of CO_2 content in blood in the course of experiment (*Leuciscus*, Acidosis Exp. 1, 5, 8).
Ordinate — CO_2 vol%. Abscissa – time in hours.
Alphabet signifies the particular individual of fish

Exp 7-9 in which the acidosis was caused by the low pH of water, showed greatly different results from the 6 experiments above

mentioned. In Exp. 7 the CO_2-content remarkably increased by 30 minutes later (in one fish it was a little delayed) quite contrary to the change of the pH which increased rapidly till 30 minutes later. This tendency was more markedly observed in Exp. 8 until 1.5 hours later, though after this the CO_2-content showed a decrease (Fig. 15).

Exp. 9 likewise showed an increase of CO_2-content till 40 minutes afterwards, excepting in a fish which showed some discrepancy.

In Exp. 10 the CO_2-content decreased by 1 to 1.5 hours later, and after that it increased. But from 24 hours afterwards is decreased with the pH. The pH in this experiment rapidly decreased until 20 minutes later, and thereafter it increased markedly. Therefore the decrease of CO_2-content until 1.5 hours later showed a discrepancy with results of Exp 7-9.

In brief, the above results suggest to us that in Exp. 1-6, in which the acidosis was caused by the O_2 want, the CO_2-content changes with the pH just as in the case of *Cyprinus*. While in Exp. 7-9 in which the acidosis was caused by the low pH of water, the CO_2-content changed inversely with the blood pH.

II) Alkalosis.

1) Alkalosis in Cyprinus. (Table 34-38, Figs. 16). In Exp. 1 the CO_2-content decreased continuously until 6 hours later, showing some discrepancy to the change of the blood pH, and this decrease was especially marked in the later half of 6 hours. Thereafter it decreased in accordance with the decrease of the blood pH. In Exp. 2, however, the CO_2-content continued to decrease until 24 hours afterwards, indicating no definite relation to the blood pH. But with regard to the pH, it markedly rose from 7 hours afterwards, and accordingly the CO_2-content changed contradictorily to the blood pH from 7 hours afterwards.

The results of Exp. 3-5 well accorded in the change of the CO_2-content. The O_2 content decreased until 5 to hours later in contradiction to the rise of blood pH. Thereafter, however, it began to increase reversely to the blood pH which tended to decrease from that time.

To sum up the above results, the relation of the change of CO_2-content to that of the blood pH appeared to be rather irregular in

Exp. 1 and 2, while in Exp. 3-5 an inverse relation was found between these two factors (Fig. 16).
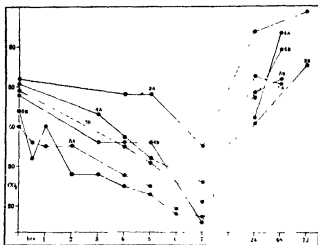
Fig. 16 Change of CO_2-content in blood in the course of experiment (*Cyprinus*, Alkalosis Exp 3 5)
Ordinate — CO_2 vol% Abscissa time in hours
Numerals attached to curves denote the No of experiment
Alphabet signifies the particular individual of fish

2) *Alkalosis in Leuciscus.* In Exp. 1 and 2 the CO_2-content decreased till from 1 to 2 hours later, and for pH change which was observed after 30 minutes no change was observed. In Exp. 3 the CO_2-content changed, in the main, with the pH change, excepting in a fish which changed the CO_2-content inversely to the pH change. In Exp. 4 the change of the CO_2-content highly varied with the individual, and each fish changed the CO_2-content irreguarly with time, showing no regularity. Exp. 5 likewise showed irregular results, and each fish changed the CO_2-content irregularly with time, though these changes agreed only in the point that all the fishes manifested the highest value after 8 hours.

In contrast to the above 5 experiments Exp. 6 showed rather a regular change in the CO_2-content. All individuals increased the CO_2-content with time, reaching the maximum at the end. But as

regards the parallelism with the pH change there seems to be no regular relation, as can be understood by referring to the distinct changes which were observed in the blood pH.

Thus we see that the change of the CO_2-content in the alkalosis of *Leuciscus* is very irregular in comparison to that of *Cyprinus*. With regard to the parallelism between the change of the CO_2-content and the pH also no definite relation was observed.

(C) RELATION BETWEEN THE pH AND CO₂-CONTENT

1) *Blood of Cyprinus.* Since the blood of *Cyprinus* buffers, like the blood of other animals, the CO_2-content under certain values of pH becomes variable. In other words the blood of a definite pH contains much CO_2 in one case while less CO_2 in another case. I found in my former investigation (26) that even in normal fishes which lived under a normal condition CO_2-content varies with the individual by some 15 vol% for the same pH. On the other hand, however, there is a loose but regular relation between the pH and the CO_2-content, which signifies that the larger the CO_2-content the higher the blood pH and vice versa. According to 36 determinations on the normal fishes in my former and present investigations the figures which represent the relation between the pH and CO_2-content fall on a narrowly limited area, designated as the normal area, of the graph. (points denoting 36 determinations have not been given in this figure as they may perplex the graph).

From the following relation a straight line is drawn which runs across the area or the centre of all the observed points (broken line in Fig. 17).

$$CO_2 \text{ vol}\% = 34. \text{ pH} - 200$$

Examining the same relation in the case of the acidosis of *Cyprinus* (116 determinations) it was found that the result of each determination is distributed somewhat irregulary and in a wider area than that of the normal case. But from all the determinations we may draw a straight line by the following equation similar to the other case mentioned above (continuous line, Fig. 17)

$$CO_2 \text{ vol}\% = 29.1 \text{ pH} - 182.7$$

By a comparison of the above two equations we notice that the
steepness of the line of normal pH-CO₂ vol% relation is a little
greater than that of the
acidosis These facts sug-
gest to us that in a nor-
mal case the blood buffers
more strongly than in the
case of acidosis

BARCROFT (9) investi-
gated the CH-vCO₂ rela-
tion (i. e CH-CO₂ vol%
relation) to the normal
human blood, and gave
the equation $vCO_2 = 8.4$
$(10^{-8} CH) + 16.6$ [i e CO_2
vol% $= 8.4 (10^{-8} CH) +$
16.6] WASTLE and SELIS-
KER (45) also studied the
same relation on the blood
of the bull frog, giving an
equation $vCO_2 = 6.0 (10^{-8}$
$CH) + 38.3$ [i. e. CO_2 vol%
$= 6.0 (10^{-8} CH) + 38.3$].

These equations bear
close resemblance to my
equation given above. But
these authers' equations
are formulated from the
CO_2 dissociation curve,
while in my case the equa-
tions were as above men-
tioned, derived empirically
from all the observed
points. Therefore it is
not of so much significance
to compare my equations
with these of the others.
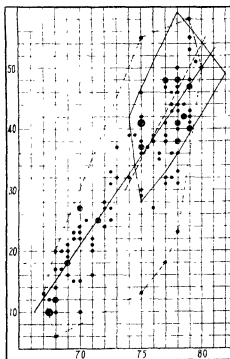
Fig 17 Comparison of normal and acido
sis areas of the pH-CO₂ vol% relation (*Cypri
nus*).

Area encircled with continued line shows
the normal area

Area encircled with broken line shows the
acidosis area.

Ordinate -- CO₂ vol%. Abscissa -- pH

Continuous slanting line shows the pH-CO₂
vol% relation in acidosis (CO₂ vol% = 29 1 pH
− 182.7).

Broken slanting line shows the pH-CO₂
vol% relation in normal condition (CO₂ vol% =
34.0 pH − 220 0)

Number of circles denotes the number of
observation repeated

As can be seen in Fig. 17 the area of normal pH-CO₂ vol% relation overlaps the area of acidotic pH-CO₂ vol% relation in the great majority of cases. But one will notice the fact that the area of acidosis is widely extended to the acid side thus indicating that the acid base balance of the blood was much shifted to the acid side.

With regard to the pH-CO₂ vol% relation in the alkalosis the observed points which represent this relation are widely scattered, so that I have not attempted to deduce an equation. Among 77 determinations of the pH-CO₂ vol% relation 52 times, i. e. 68% of the determinations, fell outside the normal area on account of the alkalosis caused by the high pH of water.

In alkalosis the pH of the blood markedly shifted to the alkali side, while the CO₂-content which was expected to be increased with the blood pH, decreased to a great extent, excepting in a few cases in which the CO₂-content increased in response to the preceding decrease. The range of the change of CO₂-content for a definite pH was much wider than that of the acidosis. But the decrease of the CO₂-content was not so marked as that of the acidosis, and rarely fell below 15 vol% (Fig. 18).

2) **Blood of Leuciscus.** The blood of *Leuciscus* also buffers though it is not so conspicuous as that of *Cyprinus*. From 53 determinations which were carried out in my former and present investigations we can form a normal area of pH-CO₂ vol% relation
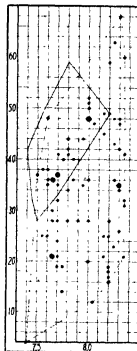
Fig 18 Comparison of normal and alkalosis areas of the pH CO₂ vol% relation (*Leuciscus*).

Ordinate — CO₂ vol%

Abscissa — pH.

Area encircled with continuous line shows the normal area

Area encircled with dotted line shows the alkalosis area

Two observations with identical values are denoted with dot encircled with circle

as drawn in Fig. 19 (Area encircled with continuous line). Although
the distribution of the figures in the normal area is much scattered,
yet we formulated the equation CO_2 vol% $= 7.9$ pH-8.42 by a com-
putation.

As is seen from the normal area pH of the normal blood of
Leuciscus varies between 7.00 and 7.70 and the CO_2-content varies
between 11 and 24 vol%. The CO_2-content varies most widely at pH
7 30, and the pH varies most widely at 18 vol% of the CO_2-content.
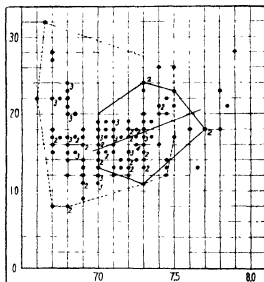
Fig. 19. Comparison of normal and acidosis areas of the pH-CO_2
vol% relation (*Leuciscus*).
Ordinate — CO_2 vol%. Abscissa — pH.
Area encircled with continuous line shows the normal area
Area encircled with dotted line shows the acidosis area.
Slanting line acrossing the normal area shows the normal pH-CO_2
vol% relation
Numerals attached to dots denote the number of observations re-
peated.

In regard to the case of acidosis this relation differs greatly from
that of the normal case. Among 120 determinations which were
made in the course of acidosis, 67 points fall outside the normal area,

thus indicating that the acid base balance is shifted to the acid side, and that the area of the distribution of each determination becomes very wide, on account of the acidosis. In the case of *Cyprinus* all the determinations fell closer to a straight line but in *Leuciscus* they were distributed very irregularly, showing even an increase of CO_2-content in the pH below 6.90. 5 points which belong neither to the normal nor to the acidotic area in Fig. 19 are the results obtained from Exp. 10. As such high pH can be regarded as a special case found in the recovery, of acidosis these were excluded from either of the normal or acidic areas.

After all, it is noteworthy that the CO_2-content in the acidosis of *Leuciscus* showed a very irregular relation for the blood pH, and showed an inclination that low pH often accompanies an abnormal high CO_2-content. For instance in Exp. 8, in which the pH was as low as 6 65, the CO_2-content was as high as 32 2 vol% Moreover for the lowest pH (6.63) (Exp. 8) of the acidosis the CO_2-content kept 21.5 vol%. But in Exp. 6 the CO_2-content was as low as 7 5 vol% for the pH 6.70 and 6.83

In general, in the case of acidosis a low pH is accompanied by a low CO_2-content, but in some cases where the free CO_2 tension of the blood s considerably high, the low pH accompanies a slightly higher CO_2-content. Therefore the low pH which is accompanied by a considerably high CO_2-content such as found in this experiment can only be explained by assuming the presence of considerably high CO_2 tension, or some other factors which will be stated in the discussion (see page 332).

In the experiment of alkalosis the pH-CO_2 vol% relation was determined 103 times Among 85 determinations (excluding 18 determinations made in normal condition) 66 times, i. e. 88% of all determinations, distributed themselves outside the normal area (Fig. 20). Though these distributions are somewhat irregular, the shape of the area of acidosis suggests possible existance of an axis which is common with that of the normal area. And hence we may be safe to assume that the pH varies proportionally to the CO_2-content, showing no such inverse relation as was seen in the acidosis.

The maximum CO_2-content in alkalosis was 32.4 vol% (Exp. 5) for which the pH was found to be 7.80. The minimum CO_2-content

was 6.0 vol% (Exp. 1) for which the pH was shown to be 6.80 to
7.00. These facts strongly affirm that the pH and CO_2 content change
proportionally to each other. In short it is remarkable that in alkalosis
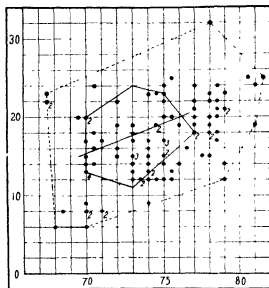
Fig. 20. Comparison of normal and alkalosis areas of the pH-CO_2
vol% relation (*Leuciscus*)
  Ordinate — CO_2 vol% Abscissa — pH
  Area encircled with continuous line shows the normal area
  Area encircled with dotted line shows the alkalosis area
  Slanting line acrossing the normal area shows the normal pH-CO_2
vol% relation.
  Numerals attached to the dots denote the number of observations
repeated.

the points which represent the pH-CO_2 vol% relation fall, in the
main, on the alkali side of the normal area. These points, however,
sometimes come to the acid side, so that the area of alkalos s becomes
very wide, thus covering the normal area (Fig. 20).

  (D) THE pH-CO_2 VOL% RELATION IN SEVERAL MARINE ANIMALS.

  The pH-CO_2 vol% relation having been stated with regard to the
blood of *Cyprinus* and *Leuciscus*, I wish to take this opportunity of

describing the same relation concerning several other marine animals with a view to summing up my present and former investigations.

The regulation of the acid base balance in blood is one of the important characters of the blood. Particularly as regards aquatic animals, in which the blood or coelomic fluid is brought into intimate contact with the external medium, this characters is worth special attention, as it may become a factor in judging the nature and extent of the adaptation of the animals to their environment.

Formerly BUNGE * suggested that the presence of NaCl in the human tissue might be inherited from some marine ancestor. QUITTON * extended this view to the blood, stating that the blood of most animals may be an altered sea water so far as the salinity is concerned. After that MACALLUM * advocated that the blood of animals might have originated from the sea water of the primitive ocean mainly for the reason that both the blood and sea water contain the same proportion of K and Ca against Na

MACALLUM further stated from this idea that the actual difference which exists between the blood and sea water of to-day or between the blood of teleosts and that of elasmobranchs may be considered as due to the changes in the composition of sea water during a long geological period, and thus suggested the possibility of an evolutional illustration of animals from a view point of physiology. Recently, S. HATAI (25) presented an account of the evolutional consideration in regard to the body fluid of animals, and threw light upon the explanation of the comparative amount of Mg contained in the body fluid and sea water, thus emphasizing the above authers' theory.

Thus in the field of comparative physiology we have seen, and will be, in future, confronted with questions which are allied to the problem above mentioned. In regard to the relation between the alkali reserve and pH of the blood, very interesting and suggestive facts which also bear on the problem of evolution, as has been suggested by the various authors mentioned above, were revealed. I have shown a graph (Fig. 21) representing the normal area of the pH-CO₂ vol% relation of five marine and fresh water fishes, and 3 marine invertebrates. In this figure the normal area of human blood

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* Indirectly cited from DAKIN (15).

which was taken from Austin and Cullen (4) is compared. One will notice from this figure that the human blood gives the highest alkali reserve and consequently the strongest buffer action. The blood of *Salmo* and *Leuciscus* follows the descending order in that regard when compared with the human blood. The fishes which are situated lowest in zoological order come close to some invertebrates such as *Caudina*, an echinodermata so far tested. Among the two molluscs, *Arca* follows *Caudina* and the Oyster is found situated in the lowest position of all.

The above order which is based on the pH-CO₂ vol% relation accords well with the zoological order; that is, the human blood is located highest and that of fishes and other invertebrates follows this. Furthermore we notice that, among fishes, the fresh water fishes such as *Cyprinus* and *Salmo* occupy the highest position, and *Leuciscus*, a brakish water inhabitant, comes next to the fresh water fish, thus showing a good agreement with the general doctrine that the fresh water animals are situated higher in the scale of evolution than the marine animals. Again, of the two marine fishes, *Pseudomonacanthus* follows the brakish water fish and *Mustelus* occupies the lowest position of the fishes.

From this order, it is also interesting to note that the brakish water fish is situated between the fresh and sea water fishes, and the primitive *Mustelus* is located just above the invertebrates. Amongst three invertebrates *Caudina* an echinodermate, came above the mollusca, likewise showing a good coincidence with the systematic order.

Dealing with this subject more in detail we found that in the normal human blood the pH changes but in a very narrow range (pH 7.30 to 7.50), while the CO₂-content varies in a wide range (55 to 74 vol%). Therefore as can be seen in Fig. 21 the normal area shows a slender form lengthwise. This means that human blood buffers well against the accumulation of carbonic or other acid, thereby preventing the pH change due to invasion of acid from without.

As for the blood of *Cyprinus* the pH and CO₂-content change in a relatively wider range (pH 7.40 to 8.20, CO₂-content 28 to 51 vol%). Consequently the normal area becomes much wider than that of the human blood. Such wide change of the CO₂-content as seen here must denote a strong buffer of the blood, provided the pH remains

unchanged or changed but a little. But as just mentioned, the pH of the blood of *Cyprinus* changes considerably, showing rather a poor buffer. This seems to be due to the fact that in the blood of *Cyprinus* the change of the free CO_2 tension is much greater than in that of the higher animals. And hence by the decrease or increase of this tension, the blood pH rises or falls in spite of the wide variation of CO_2-content.

In the blood of *Salmo* and *Leuciscus* the CO_2-content, i. e. the alkali reserve, is much less than that of *Cyprinus*. But as to the property that both the pH and CO_2-content change in a relatively wide range it is the same as *Cyprinus*.

With regard to the sea fishes, in *Pseudomonacanthus* and *Mustelus* the CO_2-content is by far smaller than that of the former species, and the changes of the pH and CO_2-content are very limited. Though these two species show no marked difference from each other in their CO_2-content, the pH is pronouncedly higher in *Mustelus*, thus showing an approximation of this species to the lower invertebrates.

Among 3 species of invertebrates *Caudina* follows the above two fishes in its CO_2-content, ranging from 7 to 11 vol%, but the pH is the highest of all, excepting *Cyprinus*, and ranges
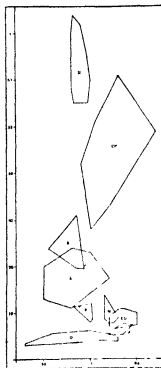
Fig. 21. Comparison of the normal areas of pH-CO_2 vol% relation of the blood of various animals. This graph was compiled from the data presented in my present and former investigations (Kokubo. 27. 28).
Ordinate — CO_2 vol%.
Abscissa — pH.
Area encircled with line shows the normal area of pH-CO_2 vol% relation.
H — Homo.   CP — Cyprinus.
S — Salmo.   L — Leuciscus.
P — Pseudomonacanthus.
M — Mustelus. CD — Caudina.
A — Arca.   O — Ostrea.

between the pH 7.70 and 8.00.

Of two molluscus, *Arca* changes its pH as well as its CO_2-content in a narrow limit, the pH ranging from 7.70 to 7.90 and the CO_2-content from 5 to 8 vol%. In the Oyster both the pH and CO_2-content were pronouncedly lower than those of *Arca*, and indeed it was the lowest of all the animals examined. The CO_2-content showed a close resemblance to that of the sea water, and changed in a narrow limit, ranging from 3 to 5 vol%. But as for its blood pH the change was very extensive, ranging in normal condition from pH 6.80 to 7.80. Therefore the normal area of the pH-CO_2 vol% relation became a slender form breadthwise. Such a form of the normal area makes a sharp contrast to the human blood, showing that the buffer action which was very strong in the human blood is exceedingly weak in the Oyster. The difference of the normal area between *Arca* and the Oyster is also of interest as seen from a biological point of view For such an animal as *Arca*, which lives in the deep muddy bottom, needs no change of the blood pH as it is surround by constant environmental factors. While in the Oyster which resides between the tide marks, it is often exposed for a long while to the air closing its shells, which forces it to cease normal respiration. As already pointed out in my previous paper (29) the pH of the blood naturally lowers in such a case. This is why the normal area of the Oyster fluctuates so widely breadthwise. Regarding the biological interest of the pH-CO_2 vol% relation, readers are referred to my former paper (29).

Thus far I have mentioned the physiological behaviour of the blood, and I shall now turn to the problem of adaptation in connection with the behaviour of the blood. In animals in which the mechanism of respiration is controlled by the central nervous system, and the respiration, in addition, is made in stable conditions, like mammalia, the acid base balance of the blood is chiefly regulated by the lung and kindney, so that the blood pH may be limited to a narrow change in virtue of the alkali reserve.

But fishes, especially fresh water fish such as *Cyprinus*, live under varied external conditions and often they are forced to live in a small mass of water under unfavourable living conditions. In addition the mechanism for the regulation of respiration seems less developed in

fishes. Accordingly it seems that the mechanism for evaporation of CO_2 or elimination of alkali is not so well provided as in mammalia. On account of such a relation the *Cyprinus* appears to be forced to alter its blood pH and CO_2-content in spite of its considerable alkali reserve. This condition may also be the case with the other fresh water fishes such as *Salmo* and *Leuciscus*, whilst in lower animals, such as invertebrates, the alkali reserve, i. e., the CO_2-content, is much less, and naturally the blood pH changes in a very wide range. Therefore it can be said that fishes resemble the higher animals in their high alkali reserve, but on the contrary they show a similarity to the lower animals in their variable blood pH. It is tempting from these facts to assume that the fishes are in the process of a transition from aquatic to terrestrial life, when considered from the behaviour of blood. If this assumption is granted then the fact that in the sea fish the CO_2-content is relatively poor and the change of the pH and CO_2-content is rather limited may be explained as being due to the reason that these fishes have not yet started the course of evolution from sea to fresh water. Hence the alkali reserve of the blood might not yet have been increased in the sea fishes.

Of three invertebrates examined *Caudina* lives in the sandy bottom of the neighbourhood of the low tide mark and its habitat is often exposed to the air. It appears to be an adaptation for such environmental conditions that *Caudina* is furnished with a relatively rich alkali reserve in comparison with *Arca* and the Oyster. Finally, in the Oyster we find a significant interrelation between the blood alkali reserve and its habits, as the Oyster lives at the tide mark and must often endure air exposure for a considerable time.

Therefore the Oyster must prevent the lowering of blood pH due to anoxaemia, by the alkali reserve. Yet the alkali reserve of this animal is very scant as can be seen in Fig. 21. This serious want of alkali is made up for by another great stock of alkali, i. e., the shell carbonate of the animal. The significance of this alkali reserve to the adaptation of the Oyster, is stated in detail in my previous article (29). After all an existence of intimate interrelation between the degree of the alkali reserve and the habitats of the animal may be explained from the above mentioned statements When the aforementioned animal are arranged according to their alkali reserve the

resulting order suggests strongly the gradual transition of animals
from aquatic to terrestrial life.

Homo. Cyprinus Salmo. Leuciscus. Pseudomonacanthus Mustelus Caudina Arca. Oyster.

Land←Fresh Water←Brakish water   ←——   Sea Water
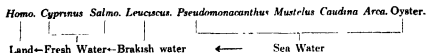
(E) CONCLUSION

(I) Acidosis in *Cyprinus*.

  1) The CO_2-content of the blood of *Cyprinus* decreases with the
fall of the blood pH. The mode of change of the CO_2-content
accords, in the main, with the pH change

  2) The CO_2-content which was decreased by the acidosis increases
reactionarily and suddenly, when the fishes are transferred to
the normal condition. (Exp. 3–5) (Fig. 16).

  3) In the acidosis of fishes the CO_2-content often changes contra-
dictorily to the pH change. This is probably due to the high
or low tension of free CO_2.

  4) The lowest limit of CO_2-content due to acidosis was 5.7 vol%
(Exp. 5) during survival, but decreased to 3.7 vol% after death.

  5) The pH-CO_2 vol% relation of the blood in normal and acidotic
conditions can be represented by the following equation (Fig.
17).

$$CO_2 \text{ vol}\% = 29.1 \text{ pH} - 182.7 \text{ (Acidosis)}$$
$$CO_2 \text{ vol}\% = 34.0 \text{ pH} - 200.0 \text{ (Normal)}$$

  6) The area of the pH-CO_2 vol% relation is shifted to the acid
side by acidosis, and enlarges its area at the same time. (Fig.
17).

  7) The CO_2-content of the normal blood is now found to be 44.83
vol%, by summing up my present and former (28) investigations.

(II) Acidosis in *Leuciscus*.

  1) The CO_2-content of the blood of *Leuciscus* is markedly changed
by acidosis. The mode of this change varies with the cause
of the acidosis.

  2) In the acidosis due to O_2 deficiency of water, the CO_2-content
decreases with pH (Exp. 1–6), while on the contrary, in the

acidosis due to the low pH of breathing water the CO_2-content increases (Exp. 7–9).

3) The lowest limit of the CO_2-content due to acidosis is 8 vol% so long as the fish survives (Exp. 6) but still further decreases to 7.5 vol% after its death.

4) After the acidosis which is caused by the acid water, the CO_2-content of the blood suddenly increses when the fish is again subjected to the respiration in natural conditions (Exp. 10).

5) In the blood of normal *Leuciscus* the pH-CO_2 vol% relation can be expressed by an equation " CO_2 vol% = 7.69 pH – 38.42 ". Such linial relation seems not to be the case with the acidotic condition, as an inverse relation is shown between the pH and the CO_2-content under the range below pH 6.90 (Fig. 19).

6) The area of the pH-CO_2 vol% relation is shifted to the acid side by acidosis and consequently enlarges its area- (Fig. 31).

(III) Alkalosis in *Cyprinus*.

1) The CO_2-content of the blood of *Cyprinus* decreases with the rise of the pH which is caused by alkalosis.

2) The decrease of CO_2-content due to alkalosis is followed by an abnormal and sudden increase of the CO_2-content, when the fish is again subjected to normal conditions.

3) The lowest limit of the decrease of CO_2-content due to alkalosis is 4.5 vol% (Exp. 1), and the highest limit, which was reached at the time of recovery, was 68.4 vol% (Exp. 3).

4) In the alkalosis the pH-CO_2 vol% relation becomes somewhat irregular, although both change, in the main, proportionally to each other.

5) The area of the pH-CO_2 vol% relation markedly shifts to the acid side, increasing its area at the same time (Fig. 18).

(IV) Alkalosis in *Leuciscus*.

1) The change of the CO_2-content of *Leuciscus* due to alkalosis is rather irregular for the change of blood pH (Fig. 20).

2) The lowest limit of the decrease of CO_2-content due to alkalosis is 6. vol%, and the highest limit due to recovering increase is 32.4 vol%.

3) The area of the pH-CO_2 vol% relation is shifed to the alkali side by alkalosis, enlarging its area at the same time (Fig. 20).

(V) A comparison of the alkali reserve of several marine animals
    suggests to us the degree of the adaptation of these animals for
    environment, and its significance to a provable evolutional course
    from marine life to inland life is discussed.

CHAPTER III.

GENERAL PART 3. BEHAVIOUR OF FISHES

The behaviour of fishes during the experiment was carefully
observed, since it is intimately related to the experimental results.

1) BEHAVIOUR OF *CYPRINUS* The general habits of this fish are
well known. When, however, this fish was placed in the experimental
jar it was excited by the change of water temperature, pH, and other
unfamiliar surroundings. But from 10 to 15 minutes afterwards it
gradually became adjusted to the new condition, breathing quietly on
the bottom of the jar. Then it swam normally around the vessel, but
with the decrease of O_2 in the water it floated and breathed the
surface water. If the O_2 was further decreased, the fish exhibited
gasping, and gill movements and its reflex became marked. With the
lapse of time the fish weakened and became agonized, shaking its
head convulsively and sometimes springing out of the vessel. There-
after it lay on the bottom, breathing first irregularly and then inter-
mittently, and finally died.

(A) Acidosis and Susceptibility.

It is obvious that the weakness of fishes which was observed during
the experiments might be due to the acidosis. But the abnormal pH
per se also may have injured the animals to some extent.

The resistance of fishes to the O_2 deficiency of water varies with
the individual, and physiological or external conditions. However the
fishes tended to float on the water surface when the O_2 decreased
below 3 cc per litre. When the O_2 became 1 cc or less the fishes
gasped, indicating an respiration, and died with a further decrease of
O_x. With the pH as low as 3.7 to 3.90 the fish survived without
any fatal effect.

As for the relation of blood pH to death and recovery, it was
found that the fish showed some debility as the blood pH became

pH 7.15. With the further weakness of fish the pH often lowered to 6.80. Even from such low blood pH the fish can yet recover, but if the pH decreases still lower it becomes unable to recover. As will be seen in the annexed table, the blood pH showed a mean value of 6.63 (mean of 6 observations) when the fishes died. The CO_2 content also showed a like relation, giving about 20 vol% when the fish were weakened and 10% or thereabout after death.

TABLE   11.

No. of Exp	Condition of fish	Blood pH	CO_2 vol%
1	Weakened, but recovered	6.90	17.5
2	Vigorous	7.25	31.0
3	Weakened, but recovered	7.15	24.7
4	,, ,, ,,	7.00	22.8
5	Died	6.00	5.7
6	,,	7.00	10.0
7 A	,,	6 80	11.9
,, B	,,	6.80	7.8
,, C	,,	6.70	12.9
,, D	,,	6.75	9.8
,, E	,,	6.75	11.9
8	Weakened, but recovered	7.53	12.7
9	,,	6.80	13.9
11	,,	6.90	19.9
12	Normal.	7.45	39.3

(B) Alkalosis and Susceptibility.

It was noteworthy, in the first place, that the alkali water was so stimulating for fishes that they needed 1.5 to 3 hours to become adjusted, while to acid water they became adjusted within 20 minutes. Secondly, in alkalosis the fishes began to lose their strength as late as 6 or 7 hours after the beginning of the experiment. This showed a marked contrast to the effects of acidosis, in which the fishes were weakened within 1 hour Thirdly, in the experiment of alkalosis the fish seldom died, while in the case of acidosis many fishes died. From these facts we notice that the alkali is more of a stimulus than the acid at first, but as to ultimate effect, the acid is more serious

than the alkali. Moreover we can say from the above result that
Cyprinus is less resistant to acidosis than to alkalosis. It may also
be said that the fishes were less weakened by O_2 want in alkali water
than in acid water, because many more fishes died in acidosis than in
alkalosis, despite the fact that the experiments were made equivalent
in regard to the decreasing O_2 of water.

Regarding the reason why the alkali water was more favourable
than acid water we may give the following two suggestions. First,
the blood pH rose in alkalosis and dropped in acidosis, while the
CO_2-content alike decreased in both conditions. Therefore we are
led to consider that the factor which unfavourably affected the fish
may have been the lowered blood pH. Secondly, the fact that
alkali water is more favourable than acid water seems to hint the
general rule that protoplasmic alkalinity favours oxydation while acidity
retards it.

The relation of the condition of fishes to the pH and CO_2-content
of blood is shown in the following table.

TABLE   12.

No. of Exp	Condition of fishe	Blood pH	CO_2 vol%
1	Died.	8.00	4.5
2 A	Recovered	8.15	21.0
B	,,	8.05	12.0
3 A	,,	8.30	34.8
B	,,	8.35	20.9
4 A	,,	8.25	15.9
B	,,	8.35	26.1
5 A	,,	8.23	16.9
B	,,	8.25	15.9

II) BEHAVIOUR OF *LEUCISCUS*. *Leuciscus* is anadromous in nature
and can live in fresh water as well as in sea water. This fish shows
no indication of injury even when it is transferred from sea to fresh
water, or vice versa.

I once made an observation, transferring 3 specimens suddenly
from sea to fresh water. These fishes seemed to feel no agony or

injury as a result of this transfer. But it was remarkable that they sank readily to the water bottom because of the sudden decrease of bouyancy. Until 1 hour later they were incapable of getting afloat by movement of the fins. After that, however, they floated and recovered their normal state in 2 hours.

The behaviour of fishes during the experiment varied with the experimental conditions. But in general they behaved more or less roughly when they were removed to the experimental jar. From 5 to 15 minutes afterwards they became quiet, and floated after from

TABLE 13.

No. of Exp.	Fish	Condition of fishes	Blood pH	CO₂ vol%
1	A	Died	7.00	12.3
	B	Much weakened, died after blood taking.	7.30	13.9
	C	,,	6.90	12.8
2	A	Left in a dying condition.	7.00	18.6
	B	,,	6.90	17.6
	C	,,	7.00	15.7
	D	,,	7.00	16.7
3	B	,,	7.03	15.2
	C	,,	7.05	19.0
4	A	,,	6.90	12.0
	B	,,	7.00	12.0
	C	Died.	6.90	11.0
5	B	Left in a dying condition	6.75	16.7
	C	,,	6.90	15.7
6	A	,,	6.90	9.0
	B	,,	6.80	8.0
	C	,,	6.70	7.5
7	A	,,	6.80	15.2
	B	,,	6.70	22.1
	C	,,	6.70	22.1
8	A	,,	6.65	27.4
	B	,,	6.70	17.6
	C	Died.	6.63	21.5
9	A	Left in a dying condition.	6.85	19.7
	B	,,	6.80	19.6

20 to 40 minutes. After gasping or making air respiration for a while, they became weakened from 30 minutes to 1 hour afterwards, and died after 2 to 4 hours. In Exp. 5 and 6, which were carried out using fresh water, the weakness of fishes was first observed as late as 1.5 hours afterwards, thus showing an appreciable distinction from the other experiments.

(A) Acidosis and Susceptibility. In the acidosis of *Leuciscus* the fishes seldom recovered. The pH and the CO_2-content of the blood after death or while dying is listed in the Table 13.

As can be seen in the above table the lowest pH (6.63) was found in a dead fish of Exp. 8, and the highest value (pH 7.30) was determined on a weakened fish in Exp. 1. The mean pH value of 25 acidotic fish was found to be 6.89. Among these 25 determinations 3 dead fish showed this value to be 6.85 in mean, indicating a slightly lower value than that of the weakened fishes. From these results we may conclude that the critical pH which fatally affects the fish is in the neighbourhood of 6.86. But under the condition of alkalosis (Exp. 6) some exceptions must be admitted, because in this case 2 fishes recovered their vigour from as low a pH as 6.75.

As already stated, the critical and postmortem blood pH of *Cyprinus* was 6.80 and 6.63 respectively. Comparing these values with those of *Leuciscus* we may say that *Leuciscus* is less resistant to the low blood pH than *Cyprinus*. As for the Oyster, which was studied in my previous work (29) the critical pH was as low as 5.00.

Hence the fatal blood pH for these animals can be designated as follows.

> *Leuciscus hakuensis.*   pH 6.86
> *Cyprinus carpio.*   pH 6.80
> *Ostrea circumpicta.*   pH 5.00

Next, with regard to the CO_2-content, the mean value of 25 individuals was 16 vol%, and the lowest value (7.5 vol%) was found in the fish C of Exp. 6, which was almost in a dying condition. While the highest value 27.4 vol% was determined on the fish A of Exp. 8, which was also in a dying condition. Among 25 determinations 3 specimens which were examined immediately after death showed the mean value to be 14.9 vol%. This value was just equivalent to 87% of the normal value. Therefore it can be said that *Leuciscus*

will be fatally affected if the CO_2-content decreases below 16 vol%, i. e. 87 vol% of the normal value.

In the acidosis of *Cyprinus*, however, the fish showed weakness when the CO_2-content became 20 vol%, i. e., 44% of the normal value, and died after reaching 10 vol%, i. e., 22% of the normal CO_2-content. Comparing these relations to those of *Leuciscus* we notice that *Leuciscus* is by far less resistant to the decrease of · alkali reserve than *Cyprinus*.

(B) Alkalosis and susceptibility. *Leuciscus* was not so markedly stimulated by alkali water as *Cyprinus*, and behaved almost equally either toward acid or toward alkali. But as to susceptibility, *Leuciscus* also showed, like *Cyprinus*, more resistance to alkali water than to acid water. Indeed in the experiment of alkalosis most fishes recovered their activity while in acidosis nearly all the fishes died. The

TABLE 14.

No. of Exp.	Fish	Blood pH		CO_2 vol%		Condition of fish
		Maximum	After death	Maximum	After death	
1	A	7.55	7.00	13.2	8.0	All died
	B	7.53	6.80	14.5	6.0	,,
	C	7.20	6.85	15.7	8.3	,,
2	A	7.35	7.25	15.0	10.9	,,
	B	7.35	7.05	19.9	11.5	,,
	C	7.31	7.05	16.8	11.5	,,
3	A	7.80		18.1		Recovered
	B	8.07		24.2		,,
	C	8.10		19.3		,,
4	A	7.43	6.97	17.3	17.3	1 died, 2 recovered.
	B	7.50		14.1		,,
	C	7.92		21.2		,,
5	A	7.90		20.3		Recovered
	B	7.80		18.8		,,
	C	7.87		21.3		,,
6	A	7.53		16.6		,,
	B	8.15		24.7		,,
	C	7.73		18.4		,,

relation of the condition of fishes to the blood pH and CO_2-content
is listed in the Table 14.

As will be seen in the above table all the fishes raised the blood
pH above the normal value. But in the fishes which were dead by
the end of the experiment, the blood pH and CO_2-content likewise
decreased abnormally after death. Thus we know that the blood
pH and CO_2-content show a rapid decrease as soon as the fish dies.
In the fish which recovered, the rise of blood pH during alkalosis
was especially marked, showing sometimes as high a pH as 8.15.
Therefore we may infer that *Leuciscus* would not be affected fatally
by a blood pH of 8.15 or thereabout, provided such pH lasts but a
short while. The CO_2-content showed an almost normal value, except
in the dead fishes in which an apparent decrease was observed. As
no particularly high CO_2-content was observed the fishes may not be
affected by it to any appreciable extent.

Among three experiments (No. 1, 2, 4), in which the fishes were
fatally affected, No. 2 showed relatively low blood pH notwithstanding
the extremely high pH of breathing water (pH 11.00). This seems
to suggest to us that the extraordinarily high pH (11.00) affected the
fishes *per se* before it caused alkalosis. Therefore we may conclude
that the pH in the neighbourhood of 11.00 fatally affects the fishes.

III) FREQUENCY OF RESPIRATORY MOVEMENTS   The frequency of
respiratory movements attracted our attention as an index to signify
the respiration stamina of the fishes. The respiratory movement varies
not only with the species but also differs according to the water
temperature, the movement of the fishes, the O_2-content of the water,
and the individuality, and vigour of the fishes. As the frequency is
referable to so many condition, as just mentioned, the complete
understanding of this phenomenon was considered to be very difficult.
According to BABAK's (5) citation of M'KENDRICK's results, *Leuciscus
phoxinus*, *Leuciscus vulgaris*, and *Cyprinus gebelis*, each measuring
from 2 to 4 inches, show the extreme frequency (per minute) to be
120, 72, and 40 times respectively.

Moreover the same author cites from P. BERT's result that speci-
mens of *Cyprinus* which measured 1.3, 37, and 120 gms indicate a
frequency of 92, 35, and 8 times respectively, thus hinting a relation
of frequency to the size of the fishes. With regard to the Japanese

Cyprinus the knowledge of this relation seems to be very meagre.
MASUGI (33) observed the respiration movement of *Cyprinus*, by
perfusing the respiratory center with a saline solution. His result
shows that fishes measuring from 380 to 570 gms. show a mean fre-
quency of about 70 times per minute, though it ranged between 45
and 128 times. YOSHIDA (45) made a similar observation on Japanese
Cyprinus. His data show that the frequency of the fishes measuring
300 to 600 gms. ranges from 40 to 70 times per minute.

Though all the above observations were made on the same or
allied species to my own, these authors, to our regret, did not observe
the water temperature, so that it is relatively of little significance, to
compare my results with theirs.

(A) Respiratory Frequency of *Cyprinus* in respect to Acidosis and
Alkalosis.

In the present study the respiration frequencies were observed in
all cases of acidosis and alkalosis. Although these observations were
made under experimental conditions, the change during the experiment
may be inferred from the data enumerated in the following table.
The water temperature during the experiment varied in a considerable

TABLE 15.

Acido- sis Exp. No.	Relation between blood pH and resp. freq	Change of Blood pH	Resp. freq. (per min.)		Water temp. (C°)	
			Mean	Range	Mean	Range
1	Resp. freq. decreased with pH.	6.90–7.95	57	48– 65	14°	11.8°–15.2°
2	No relation between pH and resp. freq.	7.25–7.95	47	32– 61	12°	10.0°–12.2°
3	,,	7.15–7.90	42	20– 53	11°	10.0°–12.2°
4	,,	7.15–7.90	73	36– 97	23°	10.5°–26.3°
5	,,	7.00–7.75	79	30–103	23°	10.3°–26.2°
6	,,	5.80–7.80	81	39–105	20°	6.3°–30.5°
7	,,	6.90–7.50	59	54– 80	13°	4.5°–23.1°
8	Resp. freq. decreased with pH.	6.70–7.87	67	60– 80	16°	4.1°–16.0°
9	No relation between pH and resp. freq.	6.85–7.87	60	47– 81	15°	4.0°–15.8°
10	,,	6.90–7.50	58	45– 62	20°	8.7°–20.2°
11	,,	6.90–7.91	56	33– 74	11°	5.0°–15.0°
12	,,	7.45–7.70	54	40– 60	14°	12.5°–16.5°

range, therefore the range of its variation is also given in the above
table and mean temperature only designates the temperature which
dominated the duration of experiments.

As can be seen in the above table, Exp 1 and 8 showed that
respiratory frequency decreases with the blood pH. In Exp. 2 and 3
the frequency was dominated only by the water temperature, and has
nothing to do with the blood pH. In Exp 4, 5 and 6 the minimum
blood pH was observed from 2 to 5 hours afterwards, while the
maximum respiration frequency was shown after from 20 to 30
minutes, thereby indicating that there is no parallel relation between
these factors   Exps. 7, 10, 11 and 12 showed no regular relation of
the respiration frequency to the blood pH.

Therefore it may be safe to conclude that there is no regular
relation between the blood pH and the respiratory frequency as far
as the present study is concerned. This well coincides with BARR's
(10) opinion that the blood reaction is not the predominant factor in
the control of breathing.   And further it supports GESELL's (19)
statement that the activity of the respiratory center is not in direct
relation to the H ion concentration of the arterial blood.

L. J. HENDERSON (26) also states that 'The theory which assumes
a single stimulus (blood pH) seems today too simple; it assumes a

TABLE 16.

Alkalosis		Relation between blood pH and resp freq	Change of blood pH	Resp. freq. (per min)		Water temp (C°)	
Exp No.	Fish			Mean	Range	Mean	Range
1	A	No relation between pH and resp. freq	7.40–8.00	61	33–77	15.0°	8.3°–16.7°
2	A	,,	7.50–8.15	55	32–67		
	B	,,	7.30–8.05	53	26–67	15.0°	7.0°–17.7°
3	A	Resp. freq. normal when the pH was highest	8.00–8.35	52	25–60		
	B	,,	8.20–8.35	49	22–60	11.0°	4.6°–12.0°
4	A	,,	7.95–8.35	39	24–58		
	B	,,	7.57–8.27	44	22–60	11.5°	7.0°–12.5°
5	A	Resp. freq indifferent to pH rise	7.63–8.23	47	24–59		
	B	,,	7.67–8.25	44	23–55	11.5°	5.0°–12.0°

process lacking in flexibility, which overlooks or at least seems to disregard the dependence of breathing upon a great many factors and the organic harmony which subsists among them '.

Consequently it follows that the general principle that the H ion is the sole exciting agent of the respiratory center can no longer be tenable.

TABLE 17.

Acidosis		Relation between blood pH and resp freq	Range of the Change of blood pH	Resp. freq. (per min.)		Water temp. (C°)	
Exp. No	Fish			Mean	Range	Mean	Range
1	A	No parallelism between pH and resp freq	7.00-7.35	72	51- 94	15.0°	13 8°-17.0°
	B		6.90-7.25	84	38-103		
	C		7.00-7.30	65	48- 80		
2	A	Highest frequency does not agree with the lowest pH.	7.00-7.42	84	56-105	11.5°	11.0° 12.2°
	B		6.90-7.45	92	68-107		
	C		7.00-7.27	90	77-102		
	D		7.00-7.50	83	55-115		
	E		7.26-7.30	78	67-107		
3	A	Ditto	7.28	88	88	11 0°	10.5°-10.8°
	B		7.03-7.20	79	58- 96		
	C		7.00-7.40	83	50-100		
4	A	Ditto	6.90-7.32	77	50- 94	11.0°	10.5°-11 5°
	B		7.00-7.18	82	81- 98		
	C		6.90-7.20	78	55- 95		
5	A	Ditto	7.06	100	100	12.0°	11.0°-12.6°
	B		6.70-7.50	77	58- 97		
	C		6.90-7.42	76	48- 91		
6	A	Ditto.	6.90-7.27	91	64-111	12.5°	12.2° 13.5°
	B		6.80-7.22	88	55-111		
	C		6.70-7.25	84	40-103		
7	A	Decrease of frequency paralleled with lowering of pH.	6.80 7.22	66	55-114	13.0°	12.6°-13.4°
	B		6.70-7.10	72	40- 58		
	C		6.70-7.22	86	45-103		
8	A	Ditto	6.65-7.95	95	50-120	13.0°	12.7°-13.5°
	B		6.70-7.00	90	77-100		
	C		6.63-7.12	76	48- 94		
9	A	Ditto.	6.55-7.15	87	58- 94	12.0°	11.9°-12.1°
	B		6.80-7.20	83	76- 90		
10	A	No relation between resp. freq. and pH.	6.90-7.87	81	56- 97	11.5°	11.0°-11.6°
	B		6.85-7.65	80	73- 91		
	C		6.70-7.85	82	69-100		
	D		6.86-7.90	92	81-100		

As for this relation in the experiment of alkalosis the above data have been obtained (Table 16).

Like acidosis, alkalosis also failed to show any parallelism between the frequency and the blood pH.

(B) Respiratory Frequency of *Leuciscus* with respect to Acidosis and alkalosis.

A marked distinction of the respiration of *Leuciscus* from that of *Cyprinus* consists mainly in its higher respiratory frequency. The mode of the change of frequency, however, also differs greatly in two species.

The relation of respiratory frequency to the change of blood pH was observed, as designated in the Table 18.

As will be seen from the Table 18 the decrease of blood pH in *Leuciscus* never increases the frequency of respiration. In Exp. 1–6 the frequency increased after from 5 to 60 minutes, though it decreased gradually thereafter. In Exp. 7–9 the respiratoy frequency decreased from beginning to end, suggesting the decrease of frequency with the lowering of pH. In Exp. 10 no regular relation was found between the frequency and the blood pH.

The increase of frequency within 1 hour of Exp. 1–6 may be due to the O_2 decrease of water. The decrease of frequency after that in the same experiments, and in Exp 7–8, may be attributed to the weakness of the fishes due to the O_2 deficiency. The reason why the frequency decreased but very slightly in Exp. 9 may be considered as being due to the slow decrease of O_2 in this experiment. In summarizing the above description we can infer that the main cause which increases the respiratory frequency of *Leuciscus* is ascribable to the O_2 deficiency in breathing water.

Finally, in Exp. 10, the blood pH was highly raised after being extremely lowered in the beginning, while the O_2 did not decrease to any appreciable degree. Therefore if the respiratory center responds sensitively to the change of blood pH it should ,exhibit some marked reaction against such an extent of pH change. But the fishes showed no response even to such a remarkable change of blood pH. This fact accurately demonstrates the absence of any relation between the blood pH and respiratory frequency.

As regards alkalosis the following data have been secured.

TABLE 18.

Exp. No.	Fish	Relation between blood pH and resp. freq.	Range of the change of blood pH	Resp. freq. (per min.) Mean	Resp. freq. (per min.) Range	Water temp. (C°) Mean	Water temp. (C°) Range
1	A	No relation between resp. freq and pH.	7.00-7.55	89	50-115	12.0°	11.9°-12.5°
	B		6.80-7.53	76	46- 95		
	C		6.85-7.20	89	53-105		
2	A	Ditto	7.02-7.35	78	41-100	11.7°	11.5°-11.7°
	B		7.01-7.35	67	37- 83		
	C		7.05-7.31	84	64-111		
3	A	Ditto	7.05-7.80	131	56-150	13.4°	13.1°-13.7°
	B		7.50-8.07	98	79-120		
	C		7.30-8.10	94	73-113		
4	A	Resp. freq. makes no change while blood pH markedly changes	6.97-7.43	88	82- 91	13.3°	13.1°-13.4°
	B		7.01-7.50	98	83-120		
	C		7.00-7.92	83	80-100		
5	A	Ditto	7.20-7.90	91	73-111	13.4°	13.3°-13.0°
	B		7.20-7.83	104	97-113		
	C		7.27-7.00	94	87-111		
6	A	Ditto.	6.75-7.53	100	46-120	13.7°	13.6°-14.2°
	B		6.75-8.15	97	75-115		
	C		6.75-7.73	92	60-111		

In the alkalosis of *Leuciscus* also, the change of blood pH has nothing to do with the respiratory frequency. In Exp. 1 and 2 the respiratory frequency decreases with time but shows no relation to the increase of blood pH. The decrease of frequency in these cases may be due to the weakness of the fishes, as neither the O_2 nor the temperature showed any decrease. In Exp. 3 the respiratory frequency showed no change, probably because of no O_2 decrease and no weakness of the fishes. That the decrease of frequency may largely be due to the weakness of fishes, can be verified by comparing the results of Exp. 2 and 6, other factors being considered as inert.

Exp. 2	O_2 unaltered	Fish weakened	Frequency decreased
Exp. 6	O_2 unaltered	Fish vigourous	Frequency unaltered

(C) **Respiratory Frequency of *Cyprinus* and *Leuciscus*.** As already mentioned the respiratory frequency of fishes varies according to many

factors, so that the experimental conditions must be unified if comparable results are hoped for. Though the present observation have been made under varying conditions, the results will sufficiently meet the purpose of gross comparison As can be seen from the table of each experiment (Table 21-51) the frequency which was listed in the table has been observed on *Cyprinus* measuring about 30 cm. in length and about 500 gms in weight, and *Leuciscus* measuring about 35 cm in length and about 500 gms. in weight.

1. *Cyprinus.* *Cyprinus* showed no change of respiratory frequency with the change of internal conditions, such as blood pH or CO_2-content, and likewise was very little affected by the change of external conditions, i. e., O_2 deficiency, pH change. or even by the weakness of the fish itself. They showed very sensitive response only to the change of the water temperature. Therefore the data obtained in the experiment of acidosis or alkalosis may be regarded as normal if it is assumed that all the factors except temperature have no relation to the respiratory frequency. In the experiment on *Cyprinus* the determination of respiratory frequency was made as often as 304 times in all. The results can be tabulated as follows

TABLE 19.

	Water temperature (C°)																							
Mean resp freq	25	26	39	41	42	45	49	47	51	48	55	57	58	64	64	—	71	64	66	47	—	82	77
Range of variation	23 22	39 39	26 22	39 29	20 32	48 40	47 55	60		49 46 67		47	50 73											
	26 28	40 45	55 65	59 60	60 61	67 70	81 80	67		105 80 76		47	105 80											
No of observation	6	9	3	3 16	11	8 18	44 45	14 17	20 22	5	—	16	8	4	1	—	13	2						

As can be seen in the above table the observation was made under a range varying from 3° to 26°C The respiratory frequency showed a considerable range of variation even under the same water temperature. But the mean frequency showed a paralleled increase with the rise of water temperature, even though some slight discrepancies were found. If the mean frequency is calculated from the above table so as to indicate the frequencies at temperatures of 5°, 10°, 15°, 20°

and 25°, the Q_{10} may be found to range from 1.41 to 1.51.

2. *Leuciscus*. *Leuciscus* manifests highly different conditions in this regard The respiratory frequency of this fish varies not only with the water temperature but also changes with the O_2 content of the water, and further according to the weakness of the fish. Accordingly all the observations made under the experimental conditions can not be taken as normal values. Therefore in the present case only the initial observation in each experiment was taken as normal value, because at the start of each experiment the fishes probably remained as yet almost unaffected by the experimental conditions. Fifty observations thus made showed the following results.

TABLE 20.

	Temperature (C°)			
	10°	11°	12°	13°
Mean no	83	96	101	109
Range of variation	78-87	82-107	86-114	91-136
No of observations	9	18	11	12

As the fishes showed some excitation when transferred into the experimental jar, the above figures may possibly shows a little higher value than the natural cases. But the increase of respiratory frequency due to the rise of the water temperature is apparent. The respiratory frequency of *Leuciscus* is about double that of *Cyprinus* under the same water temperature. This may be considered as being, in the main, due to the differences among species.

IV) CONCLUSIONS.

1) *Cyprinus* manifests weakness when the blood pH decreases below 7.15, and by further weakness the blood pH decreases as low as 6.80.

2 *Cyprinus* recovers its vigour from a blood pH 6.80; from a lower pH, however, it can not recover. The blood pH examined immediately after death is determined to be 6.63 or thereabout.

3) *Cyprinus* is more resistant to alkalosis than to acidosis, and is capable of recovering from as high a blood pH as 8.35.

4) In breathing water of high pH *Cyprinus* better resists the O_2 deficiency than in acid water.

5) *Leuciscus* is less resistant than *Cyprinus* to the decrease of blood pH, and will be fatally affected as the pH reaches **6.86**.

6) The rise of blood pH up to 8.15 has no serious effect on *Leuciscus*.

7) In susceptibility to the decrease of CO_2-content also these two species show a marked difference. *Cyprinus* is not fatally affected until the CO_2-content decreases to 22% of the normal value.

8) So far as the present study is concerned *Cyprinus* survived without injury for about 7 hours in water of pH 3.70 to 4.00.

9) *Cyprinus* also survived without injury about 7 hours in water of pH 9.10 to 9.80.

10) *Leuciscus* dies within about 2 hours when it respires in water of pH 3.4 to 3.70.

11) *Leuciscus* survives without injury about 2 hours in water of pH 9.60 to 9.70, but dies within 1 hour when the pH is raised up to pH 11.00

12) In both *Cyprinus* and *Leuciscus* the change of blood pH shows no analogy with the respiratory frequency.

13) The respiratory frequency of *Cyprinus* is controlled mainly by the water temperature. The Q_{10} of this frequency varies from 1.41 to 1 51 in the range between 3° and 26°C.

14) The respiratory frequency of *Cyprinus* is affected but slightly, by the O_2 deficiency of water while that of *Leuciscus* is affected greatly.

CHAPTER 4.

GENERAL PART 4.   DISCUSSION AND SUMMARY

I) DISCUSSION

According to Y. HENDERSON (27) the blood alkali of the higher animals shows marked accommodation to external condition. For instance the alkali reserve of human blood changes with acclimatization to altitude, and indeed in such a high altitude as 15000 feet the blood

alkali becomes only 2/3 of the normal content. Such decrease of alkali reserve, i.e., $NaHCO_3$ tends to change the blood pH which is maintained by the ratio $\dfrac{NaHCO_3}{H_2CO_3}$. But the elimination of H_2CO_3 due to physiological regulation restores this ratio and establishes a new acclimatization, thus keeping the pH relatively constant. The decrease of blood alkali in high altitude is a result of a relative decrease of the O_2 tension of the air. As the decrement of O_2 causes increased breathing and raises the pH by excessive elimination of CO_2, the blood alkali naturally decreases in order to recover the normal pH. Thus human blood manifests an unique accommodation even by a mere change of O_2 tension. Though the change of this acid base balance is occasioned by several other causes the connection of this function to the accommodation is of special interest if this relation is considered with regard to the varying species of animals.

The peculiarities which we associate with the respiration of aquatic animals are ; firstly their adaptation for the low tension of O_2 ; secondly, as the water and blood differ much in composition there is built up a steep gradient of ions and molecules across the membrane. The salts, water, gas, and protein in the blood are closely interrelated to each other, and the change of one of these factors affects all other factors (VAN SLYKE, 44). Therefore the equilibrium of substance between the water and blood may have a close bearing upon the physiology of aquatic animals. POWERS (38) stated that the ability of fishes to extract the O_2 from water of low pH depends to some extent on the pH of water, and the individual difference of O_2 absorbing ability under low O_2 and given pH may be due to the alkali reserve of the blood. PRUTHI (39), however denied POWERS' conclusion, pointing out the error of his method. Though these authors' method were ingenious, they were too simple to solve such a problem and seem to leave the question partly unanswered.

If it is granted that the affinity of the O_2 for haemoglobin varies with the pH (BARCROFT, 9) POWERS' conclusion above stated is rather probable, because my present investigations shows that the blood pH of *Leuciscus* changes with the pH of the water breathed. Consequently it follows that the pH of the water changes the affinity of haemoglobin for oxygen. Moreover POWERS (37) reported that Rock fish

changes the relative alkali reserve (pH) within a comparatively short
time, suggesting that this change may be due to the accommodation
of the fishes to the tension of CO_2 or O_2 in the water. This was
elucidated from my present study, as it has been made clear that
the blood pH of fishes changes with the O_2 tension in breathing
water. ZOETHOUT (48) studied the resistance of Paramecium to the
lack of O_2 and found that it might be increased by an addition of a
very small percentage of alkali. ANDERSON (2) reported that the
exposures of Planarians (Planaria dorotocephala) to the highly alkali-
fied water (pH 9.0) not only intensifies the rate of oxygen consumption
but also increases the susceptibility of this animal to lack of oxygen,
to toxic chemical solutions, and to ultra violet radiation. CAMPBELL
(12) also stated that the $NaHCO_3$ injected intervenously increases,
whereas HCl decreases, the O_2 consumption and CO_2 production
throughout the body. These experiments suggest to us the general
assumption that an increase in the alkalinity of protoplasm favours
oxidation while a decrease causes it to cease. PACKARD (36) made
an experiment on a fish, Fundlus heteroclitis, and found that an injec-
tion of m/15 solution of $NaHCO_3$ increases the resistance of fishes
to the lack of O_2, while the injection of acid decreases this resistance.
This also suggests the above mentioned relation of protoplasmic alkali-
nity to oxidation.

In regard to the self adjustment of fishes to the altered pH of
water BROWN's (11) work is of interest. He studied whether the fish
which live in such a high pH as 8.60 (Douglas Lake) can adapt
themselves to a pH as low as 4.40 (Vincent Lake). His study showed
that among 4 species which are common to both lakes Esox and
Ameirus adapted themselves well to the change from the acid to the
alkaline lake, but Amerius alone adapted itself easily to a reciprocal
transfer. He also showed the adaptability of some fishes to the change
from common water to this acid lake. I also observed a wide adapt-
ability of Leuciscus to the pH change. Besides these observations on
fishes the author (29) also made a study on the Oyster and found
that the change of the reaction of water or exposure of them to the
atmospheric air markedly changes the pH and CO_2-content of their
blood.

Let us now turn our attention to the results of the present in-

vestigation. Though several subjects of biological significance have been treated in the present work, the acid base balance of fish blood is the central feature of the problem. In interpreting the acid balance of the blood two main theories may be given. The simplest one is the acid poisoning theory. According to this theory the acid added to the blood is neutralized by the buffer salts of blood. The CO_2 and neutral salts thus evolved are supposed to be excreted from the lung and kidney.

$$NaHCO_3 + HCL \rightleftharpoons NaCL + H_2O + CO_2$$

Kidney   Lung

$$Na_2HPO_4 + HCL \rightleftharpoons NaCL + NaH_2PO_4$$
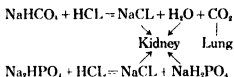
The above phenomena will really take place in some cases and will regulate the acid base balance of the blood.

But according to Y. HENDERSON's capnial theory (27) the acid base balance in the blood is also uniquely regulated by the control of respiration. For instance, when the O_2 pressure increases relatively the breathing is depressed, and consequently the lung ventilation decreases. Owing to the decrease of lung ventilation free CO_2 accumulates in the blood, and decreases the ratio of $\dfrac{BHCO_3}{H_2CO_3}$ in the following equation of L. J. HENDERSON. Accordingly the blood pH tends to fall, and to regulate this fall of pH acid is eliminated from the kidney, thus resulting in an increase of blood alkali.

$$pH = pK_1 + \log \frac{(BHCO_3)}{(H_2CO_3)}$$

FARRALORO (17) stated in his paper that, when rarefied air is breathed, the blood alkalinity decreases on account of the excessive elimination of free CO_2, but this is prevented by adding the CO_2 gas to the rarefied air. LEPPER and others (31) observed that the blood pH rises with forced breathing but it falls rapidly with cessation of forced breathing. This change of pH may also be due to the ever-poration or accumulation of CO_2 in the blood as the capnial theory explains.

Turning now to the explanation of my results regarding the acidosis of fishes, the first remarkable finding is that the O_2 decrease of water

breathed becomes an actual cause of the acidosis of fishes. I associate this fact with experiments made on the dog by HAGGARD and HENDERSON (23). These authors found that in the dog acidosis is caused by a mere O_2 deficiency, and explained the lowering of blood alkali in this case by the acid poisoning theory at first. But in a later publication they replaced the former explanation by the capnial theory, and explained the lowering of blood alkali as follows: Overbreathing blows off an excess of CO_2 (Acapnia), leaving the CO_2 ratio, i. e., the relation of H_2CO_3 to $NaHCO_3$, and therefore presumably the cH in the blood, below normal. This alkalosis is then compensated by a disappearance of alkali from the blood. Therefore, in this case, overbreathing due to O_2 deficiency becomes the sole cause of the acidosis.

Observing this relation in the acidosis which was induced in fishes by O_2 deficiency, it seems somewhat to vary with the species. In *Cyprinus* the acidosis was caused immediately after the O_2 decreased to some extent. The excitement of respiration due to O_2 deficiency in the water breathed was also observed in this fish (Acidosis Exp 2, 3, 6. 9), though it was not so marked as in the higher animals. Therefore the increment of the respiration frequency due to this experiment must affect the fish, thus resulting in overbreathing. If this overbreathing makes the free CO_2 evaporate excessively the fishes have to cause an alkalosis as in HAGGARD and HENDERSON's observation on the dog. On the contrary, however, there was produced an acidosis instead of alkalosis. This result is quite opposed to HENDERSON's capnial theory. But with regard to the *Leuciscus* much difference has been found in this connection. In this fish the increase of respiration due to O_2 deficiency was more marked than that of the *Cyprinus* (Acidosis Exp. 3, 4, 5, 6). Consequently the change of the blood pH also showed a distinct difference, giving an apparent alkalosis at first and then returning into acidosis with the depression of respiration. The alkalosis in this case may undoubtedly be caused by the excessive elimination of CO_2, and acidosis may be caused by the disappearance of alkali and the accumulation of CO_2 in the blood. Thus the capnial theory of HENDERSON was fairly applied to the acidosis of *Leuciscus*.

As above stated some excitement of respiration was observed at the beginning of the acidosis of *Cyprinus*. If this excitement acts on the animal to induce overbreathing, the result causes acidosis in place

of alkalosis, and this is contrary to HENDERSON's capnial theory. But it seems that the increase of respiration in this case was not so powerful as to eliminate an excess of CO_2 from the blood. It thus induced no alkalosis.

On the other hand the reduced haemoglobin of the blood is not fully oxygenated because of the O_2 deficiency in the water breathed. Therefore the acidity of the oxyhaemoglobin was insufficient to neutralize the bicarbonate of the blood (VAN SLYKE, 42). Thus the bicarbonate remains in the blood without giving off CO_2, and hence the CO_2 combining power of the blood decreases excessively. Accordingly the free CO_2 which was washed out from the tissue accumulates in the blood in a great amount. The carbonic acid thus evolved changes the ratio of $\frac{NaHCO_3}{H_2CO_3}$, causing the acidosis in question.

By the above explanation the acidosis in Cyprinus due to O_2 deficiency is accounted for. But according to this explanation the bicarbonate of the blood must remain unchanged, as can be seen from HENDERSON-HASSELBALCH's equation. In fact, however, the CO_2-content, i.e., the bicarbonate, was found to have been decreased by this acidosis. Therefore in admitting the above explanation we must assume a disappearance of the bicarbonate from the blood either by excretion through the kidney or by reabsorption of it to the tissue. But as this bicarbonate reappears with the recovery from acidosis the disappearance of bicarbonate may be most probably attributed to the reabsorption of alkali to the body tissue.

Regarding the acidosis which was caused in Leuciscus by O_2 deficiency a small alkalosis preceded the acidosis as the experiment started. This alkalosis in Leuciscus may be attributed to the loss of CO_2 due to overbreathing. In the acidosis which follows this alkalosis, the bicarbonate may have disappeared in order to compensate for the alkalosis. Owing to the O_2 deficiency, on the other hand, the CO_2 carrying power of the blood may be highly decreased on account of the decrease of the acidity of haemoglobin, resulting in the accumulation of CO_2. The CO_2 thus accumulated in the blood may become the direct cause of acidosis, as it changes the ratio of $\frac{NaHCO_3}{H_2CO_3}$.

With regard to the acidosis in Leuciscus which was caused by

water of low pH, the explanation must be given from the acid
poisoning theory.   In this acidosis the acid which has invaded into the
blood may have neutralized the blood bicarbonate.   The CO_2 evolved
in this case may be evaporated from gill and the neutral salte and
water may be excreted from the kidney.   The diminution of alkali
thus produced will cause the acidosis, shifting the rate of $\frac{NaHCO_3}{H_2CO_3}$
to the acid side   A remarkable feature of this acidosis consists in its
great rapidity, this may provably be due to the rapid penetration
of acid from without into the blood   In this case the alkalosis which
was found in the acidosis due to O_2 deficiency was not observed.
This may also be ascribed to the rapid invasion of acid.   Because in
this case the acid will diminish the bicarbonate before it is made to
disappear by the alkalosis.   Such rapid penetration of acid into the
blood was also observed in my study in the blood of the Oyster (29).

The fact that the acidosis is producible in *Leuciscus* and the Oyster
by acid water, while it is incapable of being induced in *Cyprinus* by
the same cause is of peculiar interest.   Such a difference, however,
may be regarded as depending on the ability to regulate the gill
membrane which enables the acid to permeate in *Leuciscus* and the
Oyster but not in *Cyprinus.*

It was observed in the acidosis in *Cyprinus* (Exp. 1-4) that the
blood pH which was lowered by the O_2 deficiency of breathing water
rose again within 24 hours, when the fishes were replaced in the
natural tap water.   This rise of blood pH can be explained as follows.
As the reduced haemoglobin becomes oxyhaemoglobin by the O_2 in-
crease of water, the CO_2 is given off from bicarbonate by the acidity
of oxyhaemoglobin and the CO_2 carrying power of blood is increased.
By this recovery of normal condition the alkali which was once
reabsorbed to the tissue at the early stage of acidosis reappears, and
increases the blood pH as well as the bicarbonate.

At any rate it may be empirically inferred that the insufficient
oxygenation of haemoglobin decreases the CO_2 combining power of
the blood alkali, and such change induces the reabsorption of alkali
from body tissue.

In the acidosis of *Leuciscus* due to O_2 deficiency also a rise of
pH was observed at the later stage (Acidosis Exp. 10).   This may

be explained as being in part due to a production of bicarbonate from tissue, and in part to penetration of the OH ion from breathing sea water.

We may now pass on to the problem of alkalosis The alkalosis in this experiment was caused by NaOH in the water breathed. If, therefore, the molecule of NaOH penetrates from without into the blood, it will combine with the carbonic acid as the following equations show.

$$H_2CO_2 + NaCH = NaHCO_1 + H_2O$$
$$H_2CO_1 + 2NaOH = Na_2CO_1 + 2H_2O$$
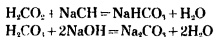
Therefore the blood pH must be increased both by the neutralization of H_2CO_2 and the increase of alkali reserve in the blood. Indeed, in the alkalosis of *Cyprinus*, the pH increased at a greater rate, but as to the alkali reserve a remarkable decrease was observed in place of an increase Accordingly we are tempted to belive that only the OH ion may penetrate into the blood, and that the disturbance of electrostatics caused by the migration of the OH ion might be equilibrated by some appropriate process. With regard to *Leucscus* the rise of the pH was also very marked, but the change of the CO_2-content did not show any regular relation in its parallelism to the pH change. Therefore whether the NaOH penetrated into the blood or not could not decided in this case. However the OH ion might undoubtedly have invaded the blood, for the blood pH would not make such a rise if the OH ion had not invaded from without. This is demonstrated by the experiments in which the blood pH rose without increasing the bicarbonate or decreasing the carbonic acid (Exp. 4 and 5).

Concerning the permeability of the vital membrane I may quote my studies which were made on the Oyster (29). In this animal the OH of water rapidly penetrates into the blood in some cases, and decreases the blood alkali, while in some other cases OH never penetrates into the blood. But at any rate it was clear that the NaOH of water dose not invade into the blood, as any increase of blood alkali was not observed. Comparing these results with those of the fish we will be aware of a considerable resemblance in the behaviour of the gill membrane. And thus I am tempted to conclude that the OH ion penetrates the gill membrane of these animals, while

the Na ion dose not penetrate. Though in this case an opposing electrostatical potential may be built up across the membrane, it may probably be compensated for by the migration of some anion, say HCO_3, from within.

The acidosis which was observed within the first five hours of the experiment in the alkalosis in *Cyprinus* may be considered as being due to the O_2 decrease of breathing water. The OH ion of water may, of course, have penetrated before this acidosis ceased but it was slow at first and it seems to have been unable to raise the pH until the OH ion overcame the acidosis.

The blood pH which was raised by the alkalosis continued its high value until 24 or more hours later. This may of course have been partly due to the long stay of the OH ion coming in from water. But it may have been partly due to the increase of bicarbonate, since it was found actually increased by that time.

As for the alkalosis of *Leuciscus* the blood pH immediately began to rise after the fish was placed in the alkali water, showing a keen distinction in contrast to *Cyprinus* in which a temporary acidosis preceded the alkalosis. This may be due to the reason that the invasion of the OH ion into the blood of *Leuciscus* was so rapid that the blood was incapable of lowering its pH. The pH thus raised continued for a long time even after the fish was removed to the tap water. This persistency of pH highly resembles that of the high pH which was observed in the alkalosis of *Cyprinus*. The reason for this may be same as that of *Cyprinus*.

As already stated the blood pH which was depressed by the acidosis rose rapidly after the fish was placed in the tap water (*Leuciscus*, Exp. 10). Comparing this with the persistent high pH produced by alkalosis it can be said that there is a marked difference between acidosis and alkalosis with regard to the speed of recovery of normal pH.

In short the permeability of the gill membrane varies with the kind of ion and with the species of fish. This can be induced from the following facts: (1) *Cyprinus* does not lower its blood pH in acid water but rises the blood pH in alkali water. (2) *Leuciscus* lowers the blood pH in acid water, and rises it in alkaline water. In *Leuciscus* the lowering of blood pH is instantaneous but the rise is

somewhat slow.

The foregoing observations were chiefly concerned with the change of the pH. In what follows I will give some brief comments in regard to the change of the CO_2-content. A remarkable feature regarding this is that the change of the CO_2 content is more or less irregular contrasted with the regular change of the pH. In consequence, the relation of pH to CO_2-content becomes somewhat intricate. Such irregularity and intricacy may chiefly result from the complex function of the regulation of respiration. And it seems to be unable to give suitable interpretation unless further detailed study were made. The decrease of CO_2-content of fish blood due to the O_2 decrease of breathing water has been more than once mentioned in the preceding pages. In higher animals alkali decrease due to O_2 deficiency was first ascribed by HAGGARD and HENDERSON (23) to the production of acid, after that, however, this explanation was replaced with the capnial theory by same authors, suggesting that the O_2 deficiency 'eliminates the blood alkali (23). Either of these theories seems to serve in explanating the alkali disappearance in my case. But these two theories likewise assume the elimination of alkali from the body, while in the present case the alkali reappears very rapidly and noticeably, so that it was considered not to be appropriate to assume the excretion of alkali from the body. Therefore I assumed the alkali to be re-absorbed to the body tissue, thus admitting of its return whenever required.

But, as regards the acidosis of *Leuciscus* which was caused by acid water (Exp. 7, 8, 9), the explanation must be altered. In this acidosis the CO_2-content increased in contradiction to the case above mentioned. The increase of CO_2-content due to acidosis is seemingly unreasonable. But as stated in my study of oyster blood (29) the soluble carbonate of the body such as $CaCO_3$ may be washed out, and may produce bicarbonate. This was very accurately observed in the acidosis of the Oyster, and hence it may not be unreasonable to apply the same explanation for *Leuciscus*. Thus I am tempted to presume that the readily soluble calcium carbonate which was deposited in the cartilage or bone may serve as the alkali reserve of the body.

In regard to the change of CO_2-content in the alkalosis of *Cyprinus* it was notable that the CO_2-content decreases 7 hours later despite

the pH increases. In this case the relation which exists between these two factors appears to be quite contradictory to the general law of acid base balance. But it may be explained as follows: the blood pH increases by slight invasion of the OH ion from the water breathed, while the CO_2-content decreases by a reabsorption of it to the body tissue because of the O_2 deficiency of the water breathed. The increase of CO_2-content which was observed from 7 hours later on was regarded as being due to the reappearance of alkali from the body tissue. Thus the disappearance and reappearance of alkali was considered as according to the same mechanism as that of acidosis. Finally, as for the change of the CO_2-content in the alkalosis of *Leuciscus* it seems to deserve no comment on account of the irregularity of the results, so no special mention is made.

II) GENERAL SUMMARY

a) Acidosis and alkalosis in fishes, which were produced under experimental conditions, have been studied. The outstanding findings of the present work can be designated as follows.

   1) It has been determined whether or not the blood pH can be altered *in vivo* experimentally, and to what extent it varies if it changes at all. The result showed that the blood pH of fishes changes *in vivo*, ranging from pH 6.80 to 8.35 in *Cyprinus* and from pH 6.70 to 8.15 in *Leuciscus*

   2) It has also been determined whether or not the CO_2-content of fish blood can be altered *in vivo* experimentally, and to what extent it varies if it changes at all. The results showed that the CO_2-content of fish blood changes *in vivo*, varying from 5.7 vol% to 68.4 vol% in *Cyprinus*, and from 8.0 to 32.4 vol% in *Leuciscus*.

   3) The difference between the above mentioned relations according to species can be comprehended from the above statements.

   4) The relation which exists between the blood pH and the CO_2-content in respect to both species can be expressed by the following equations.

$$CO_2 \text{ vol\%} = 34.0 \text{ pH} - 200 \dots \text{ } Cyprinus$$
$$CO_2 \text{ vol\%} = 7.69 \text{ pH} - 38.42 \text{ . } Leuciscus$$

5) The susceptibility and the behaviour of the fishes in regard to changes of internal medium (blood) as well as external medium (breathing water) have been determined. According to the present results, *Cyprinus* and *Leuciscus* are capable of surviving in the range of blood pH above mentioned. Resistance to the change of internal and external medium is stronger in *Cyprinus* than in *Leuciscus*. With regard to the behaviour of fishes readers are referred to the conclusions of Chapter V.

6) A general doctrine that the H ion concentration of the blood uniquely excites the respiratory center of the animal was put to the test, making use of fishes as material, and data denying this doctrine has been obtained.

7) My present and previous investigations have been summarized, comparing the pH and the alkali reserve of various marine forms, and an evolutional consideration has been given from a view point of comparative physiology. From the idea thus derived we can conclude that the difference in the alkali reserve of the blood of aquatic animals due to species may be regarded as a result of the adaptation of animals to environmental conditions, especially to the quantity of O_2 in water.

b) Detailed conclusions concerning the above enumerated items have been stated at the end of each chapter.

c) The trends shown by the present investigation suggest to us that the pH and the CO_2-content will give us many significant departures when the questions are extended to the problem of applied biology. This statement is based on the fact that various material questions which are familiar to many aquarists may be solved by the knowledge of the acid base balance of the blood of aquatic animals.

CHAPTER 5.   PROTOCOLS

Part 1, EXPERIMENT ON *CYPRINUS*

A) ACIDOSIS.

Cyprinus, Acidosis Experiment 1.
(Oct 23rd–25th, 1927)

In the present experiment the breathing water was not renewed until 7 hours afterwards, but after determining the decrease of pH and CO_2-content at the 7th hour, running water was supplied to observe the recovery of the blood pH and CO_2 content.

The fish used was four years old, measuring 28 cm in body length, 9 cm in body height, and 470 gms in body weight, and was kept in tap water for 10 days previous to the experiment

TABLE   21

Case No	Time	Air temp (C)	Breathing water Temp (C)	pH	O₂ (cc)	Blood pH	CO₂ vol%	Resp freq	Behaviour of fishes
1	0m	17.0	13.5	7.45	6.223	7 (2	39.3	54	Somewhat excited.
2	10	17.0	13.5	3.90	6.028			55	Normal.
3	20	17.0	13.5	3 90			53		,,
4	30	17.0	13.5	3.90	4.559			56	,,
5	1 h	17.0	13.7	3.90	3.941			52	Floated on the surface
6	2	17.0	13.9	4.00				62	,,
7	3	17.0	14.2	4.00	3.765			62	A little weakened.
8	4	17.3	14.5	4.10				61	Began to gasp.
9	5	18.1	14.7	4.10				63	Gasping
10	6	19.0	14.8	4.15				65	,,
11	7	20.0	15.2	4.20	3.402	6.90	17 5	61	Highly weakened
12	24	12.5	12.8	7.20	5.445	7.95	49.5	51	Recovered.
13	48	12.6	11.8	7.20	6.347	7.90	—	48	Active.

Cyprinus, Acidosis Experiment 2.
(Oct. 25–27th, 1927).

Exp. 1 suggests that the fish which respired over 7 hours in the acidified water (pH 3.90) markedly decreases its blood pH as well as

its CO_2-content, though it turned out erroneous later. A further experiment was tried to observe the rate of this decrease until 7 hours later. The determination of the pH and the CO_2-content was made 6 times in all, i. e., before the outset of the experiment, 1, 3, 7, 24, and 48 hours after the start of the experiment. Duration of the experiment, 48 hours.

The fish employed was 4 years in age, measuring 28,6 cm. in body length, 8,2 cm. in body height, and 464 gms. in body weight.

TABLE 22.

Case No.	Time	Air temp (C)	Breathing water			Blood		Resp. freq	Behaviour of fishes
			Temp (C)	pH	O_2 (cc)	pH	CO_2 vol%		
1	0m	13 0	12.0	7.10	6.060	7.70	47.7	59	Remained still
2	10	13.0	12.0	3.80				52	,,
3	20	13.0	12.0	3.80				52	,,
4	30	13.0	12.0	3.80				52	Breathe a little irregularly
5	1 h	13.0	12.0	3.80	5.308	7.70	47.7	40	,,
6	2	13.3	12.1	3.80	5.111	7.60	37.5	44	,,
7	3	13.3	12.2	3.90	2.759	7.50	40.5	48	Body became opaque.
8	4	13.3	12.2	3.95				55	Floated on the surface.
9	5	13.3	12.2	4.00	2.240	7.30	31.6	61	Breathed on the surface.
10	6	13.3	12.2	4.10				52	,,
11	7	13.1	12.2	4.10	1.141	7.25	31.0	43	,,
12	24	11.0	10.9	7.00	6.150	7.80	50.2	51	Quiet.
13	48	10.0	10.0	7.15	5.949	7.90	50.9	46	,,

Cyprinus, Acidosis Experiment 3.
(Oct. 27th–29th, 1927).

This experiment was intended in part with the object of determining the change of the blood pH which occured within 7 hours, and in part to verify the increase of pH which was observed at 23 hours or more later in the Exp. I and II. The fish used was 4 years in age, measuring 32.0 cm in body length, 8.0 cm in body height, and 470 gms in body weight.

The results observed are given in the following table.

TABLE 23.

Case No.	Time	Air temp. (C)	Breathing water			Blood		Resp. freq	Behaviour of fishes
			Temp (C)	pH	O$_2$ (cc)	pH	CO$_2$ vol%.		
1	0m	12 0°	11.4°	7.30	6.534	7.75	45.7	34	A little excited.
2	10	12.0°	11.4°	3.70	6.098			41	Normal, still
3	20	12.0°	11.4°	3.70	5.704			30	Normal, swimming.
4	30	12.5°	11.4°	3.70				20	,,
5	1 h	13.5°	11.5°	3.70	4.843			55	Breath regular.
6	2	15 0°	11.5°	3.80				48	,,
7	3	16.0°	11.5°	4.00	3.796	7.18	25 0	50	,,
8	4	17.0°	11.7°	4.00				52	,,
9	5	18 0°	12.0°	4.00				52	,,
10	6	17.0°	12.5°	4 10				52	,,
11	7	14.5°	12.5°	4.12	2.448	7.15	24 7	51	Somewhat weakened.
12	24	10.0°	10.0°	7.15	5.438	7.90	54.5	34	Recovered, active
13	48	10.0°	10.0°	6.80		7.85	53 1	32	,,

Cyprinus, Acidosis Experiment 1
(Oct 29th, 9 30 am, 1927)

The aforementioned 3 experiments show us that the blood pH and the CO$_2$-content of *Cyprinus* decreases when the animal is forced to respire for several hours in the experimental condition. Therefore I tried a further experiment to find whether such decrease can be induced more rapidly by raising the temperature of the breathing water.

Though the procedure of experiment was almost similar to that of the former experiments, the container used in this experiment was a square glas jar measuring 40 cm in length, 29 cm in breadth and 27 cm in depth. The fish employed was four years in age measuring 30.0 cm in body length, 79 cm in body height and 380 gms in body weight. The data obtained are given in Table 24.

Though the fish appeared normal at first its body colour became whitish after 10 minutes and excreted a large amount of mucus in the water. Thereafter the fish tended to float on the water surface showing a little weakness, and body colour became markedly white.

TABLE 24.

Case No.	Time	Air temp. (C)	Breathing water			Blood		Resp. freq	Behaviour of fishes
			Temp (C)	pH	O_2 (cc)	pH	CO_2 vol.%		
1	0m	10.0	25.0	7.40	6.357	7.75	42.5	50	Active.
2	10	11.3	26.3	3.90	5.889			73	Excreted mucus
3	20	11.3	26.2	3.90	5.652			80	A little weakened
4	30	11.4	25.5	3.95	5.208			97	,,
5	1h	12.0	25.2	4.10	4.024	7.15	25.0	67	Floated, gasp.
6	2	12.5	25.3	4.20				75	,,
7	3	13.2	25.4	4.35	2.209	7.05	25.0	83	Highly gasping
8	4	12.3	25.0	4.50				81	,,
9	5	11.3	25.0	4.60	1.711	7.00	22.8	85	,,
10	24	10.0	10.5	6.80	5.438	7.30	37.0	36	Recovered, vigorous.

After 1 hour the fish was much weakened and began to gasp under the water surface. 3 hours later the gasp became more severe making deep breathing and increasing the frequency of the reflex gill movement. But after returning to the fresh tap water it largely recoverd the ill effects mentioned above.

Cyprinus, Acidosis Experiment 5.
(Oct. 28th, 12.00 n., 1927).

The present experiment has been intended with the object of determining the special change of blood pH and CO_2-content due to the rise of the temperature of breathing water.

The fish employed was four years in age and measured 31.0 cm in body length, 7.9 cm in body height, and 415 gms in body weight. The data obtained were as the Table 25.

Cyprinus, Acidosis Experiment 6.
(Dec. 18th, 10.00 am., 1927)

In the experiments hitherto conducted the pH and CO_2-content were determined several times on the blood which was bled from one individual at intervals. While in the present experiment the determinations were made on 6 different individuals. Among these fishes

TABLE 25.

Cage No.	Time	Air temp (C)	Breathing water Temp. (C)	pH	O₂ (cc)	Blood pH	CO₂ vol%	Resp freq	Behaviour of fishes
1	0m	12.2	10.3	6.80	6.527	7.80	48.2	30	Quiet, breath regular
2	10	13.0	25.2	3.80	6.098			70	A little weakened
3	20	13.0	25.0	3.80				90	Body became opaque
4	30	13.0	25.0	3.90				105	Breath regular.
5	1h	13.2	25.0	3.90	4.065	6.90	15.9	100	,,
6	2	13.4	25.0	3.95	3.017			80	Weakened, sometimes lay down
7	3	13.0	25.2	4.20	2.821	6.00	5.7	80	Nearly died
8	3.5	12.2	25.2	4.20	2.800	5.80	3.7	—	Died.

5 were subjected to respire in the breathing water which showed the
pH 3.9 to 4.50 and temperature 20°C The remaining one fish was
kept in another jar supplied with tap water.

The manner of the experiment was nearly equal to those of the
former experiments. Five fishes were placed in a jar containing 20
litre of breathing water, and bleeding were made after 30 minutes,
1 hour, 2 hours, 3 hours, on each fish. The fishes, once
bled, excluded from the experimental jar one by one.

The fishes employed gave the following measurements.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	30.0	8.0	392
B	30.0	8.4	474
C	32.5	8.9	490
D	32.0	8.8	395
E	29.0	8.1	321
F	30.5	7.8	346

The results of the experiment were as follows.

TABLE 26.

Case No.	Time	Air temp. (C)	Breathing water Temp. (C)	pH	O₂ (cc)	Blood pH	CO₂ vol%	Temp. freq.	Behaviour of fishes	Fish
1	0m	10.3	6.3	7.60	6.992	7.50	46.0	39	Breathed indistinctly.	A
2	10	10.5	20.2	3.90	4.185	—	—	75	Breathed distinctly.	—
3	20	11.0	20.5	3.90	..	..	—	105	Still	—
4	30	11.0	20.0	4.00	1.682	7.50	36.0	97	Still, excreted mucus	B
5	1h	12.2	20.5	4.10	1.264	7.25	33.0	87	2 fishes afloat.	C
6	2	12.2	20.5	4.20	1.021	6.90	18.0	77	Fish A lay afloat	D
7	3	12.0	20.3	4.30	0.978	7.00	22.0	86	Still, mucus increase	E
8	4	11.0	20.0	4.40	0.978	—	—	—	,,	—
9	5	11.0	20.1	4.50	0.757	7.00	10.0	81	,,	F

Cyprinus, Acidosis Experiment 7.
(Dec 20th, 9 37 am , 1927)

In the present experiment 6 individuals were employed and the treatments were made in a similar manner as the preceding experiment (Exp. 6). The temp. of breathing water was gradually raised, after placing the fishes in the jar, to 20°C after 1 hour. Thereafter the temperature was further raised from 20.0° to 23.1° in 5 hours. The pH of breathing water was kept 3.90 to 5.40 during the experiment. Determination of the pH and CO₂-content was made 3 and 6 times respectively on one fish, and thus altogether 24 determinations were made on 6 fishes.

The fishes employed measured the following dimensions.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	31.1	9.1	522
B	31.0	8.8	515
C	30.0	9.2	529
D	29.0	8.0	455
E	30.0	8.0	521
F	31.5	9.0	540

TABLE 27.

Case No.	Time	Air temp. (C)	Breathing water		Blood			Resp. freq.	Behaviour of fishes	Fish
			Temp. (C)	pH	O$_2$ (cc)	pH	CO$_2$ vol%			
1	0m	8.3°	4.5°	3.90	8.569	7.87	48.0	34	Quiet, breath irregular.	F
2	5	8.5°	7.0°	3.90	7.591	7.79	45.7	29	A little active.	A
3	10	9.2°	10.0°	3.95	6.550	7.70	41.1	49	Quiet.	B
4	20	10.0°	12.0°	4.00	5.593	7.85	47.4	60	Breath regular.	C
5	30	10.5°	15.1°	4.00	4.311	7.85	47.4	49	Normal, lively.	C
6	1.h	11.0°	20.0°	4.20	1.314	7.75	35.5	51	Tended to float.	A
	h m									
7	1.30	12 2°	20.5°	4.30	0.678	7.35	32 2	80	All fishes afloat.	E
8	1.35	12.5°	21.0°	4 35	0.473	7.20	28.3	46	A little weakened	D
9	1 45	12.3°	21.5°	4.42	0.421	7.12	21.0	80	Afloat and gasping.	B
10	2.00	12.3°	21.5°	4.50	0.368	6.95	22.9	55	Fish E laid afloat.	E
11	2.10	12 0°	21.5°	4.60	0.515	6.95	22.0	56	All fishes weakened.	A
12	2.20	12.3°	21.5°	4.80	0.515	7.00	27.0	80	Fish C active, A much weakened	C
13	2.30	12.3°	21.0°	5.00	0.421	6.80	11.9	60	Fish A nearly died, E much weakened.	A
14	2.50	12.4°	21.0°	5.20	0.368	6.75	11.9	54	Fish D laid afloat, E nearly died.	E
15	3.00	12.5°	20.0°	5.30	0.421	6.80	9.8	55	B and D lay afloat.	B
16	3.15	13.0°	20.0°	5.30	0.452	6.90	21.1	75	Fish B weakened.	C
17	3.20	13.0°	20.0°	5.30	0.526	6 80	16.8	47	B behaved furiously.	D
18	3.30	13.0°	23.1°	5.33	0.473	6.85	16.8	57	B nearly died, D laid afloat	C
19	3.40	13.2°	22.8°	5.35	0.789	6.80	7.8	68	B died, C lay down.	B
20	4.00	13.2°	22.6°	5.40	0.736	6.80	11.9	65	C much weakened.	D
21	4.10	14.0°	22.3°	5.40	0.789	6.70	12.9	78	C died, D lay afloat.	C
22	4.30	14.2°	21.7°	5.40	0.790	6.75	9.8	75	,,	D
23	4.40	14.0°	22.3°	5.40	0.894	6.75	9.8	54	,,	D
24	5.00	14.0°	21.4°	5.40	0.946	6.75	9.8	59	Fish D nearly died.	D

The following figures will serve to show the relation between the change of experimental conditions and that of the fish blood.

The fishes used in the present experiment showed very regular change in their CO$_2$-content of the blood as will be seen from Fig. 23.
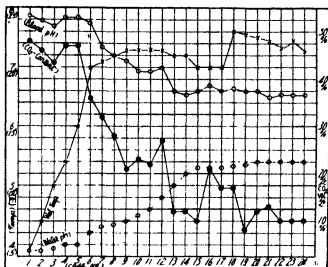
Fig 22. Change of blood pH, CO_2-content, water temperature and water pH in the course of experiment (*Cyprinus*, Acidosis Exp 7).
Ordinate (left side) — pH and temperature.
Ordinate (right side) — CO_2 vol%.
Abscissa — number of case.
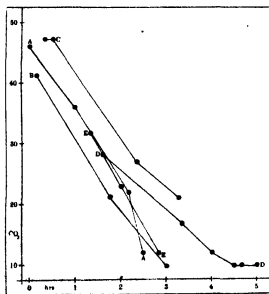
Fig. 23. Decrease of CO_2-content of blood (*Cyprinus*, Acidosis Exp. 7).
Ordinate — CO_2 vol%.   Abscissa — time in hours.
Alphabet signifies the particular individual of fish.

From Fig. 24 which shows the pH-CO$_2$ vol% relation to be a straight line, we notice that the blood of fishes directly changes its CO$_2$-content in association with the change of pH. Therefore the acidosis in fishes is socalled uncompensated acidosis contrasted with the compensated acidosis which always occurs initially in all cases of acidosis in higher animals.

Most of the fishes in the present experiment behaved normally till 1 hour later. But some fish showed a tendency to float on the water surface, and showed a little weakness.

After 1 hour and 3 quarters fish E and after 2 and half hours fish A lay afloat and came to a dying condition. After that all other fishes were equally weakened except fish C which maintained its normal vigour for another hours. 3 hours later fish A died and fishes B, D, and E lay afloat. 3 and a half hours later fish B appeared in a dying condition and fish C showed marked debility for the first time.

Fig 24 Relation of blood pH to the CO$_2$-content of blood (Cyprinus, Acidosis Exp 7)
Ordinate — CO$_2$ vol%   Abscissa — pH
Circles denote the number of observations repeated
Slanting line shows the pH-CO$_2$ vol% relation

After 4 hours all fishes died except C and D, though the former fish C, died after 5 hours. Fish D, which remained in fatal codition to the last, also died after a short while.

As listed in the table, the O$_2$ content of breathing water showed the initial value to be 8.6 cc per litre. 30 minutes later it became about 50% of the initial content and further decreased down to 15.%

of the initial content 1 hour later.

After 1 and a half hours it became 6.5% and at last it reached 0.37 cc, i. e., 4.3% of the initial content giving the minimum quantity. From two hours onwards till 3 and a half hours later an equilibrium was built up between the oxygen quantities which are consumed by fishes and the quantities which dissolved from atmospheric air. And hence there was no change in oxygen quantity. But after that the oxygen which diffused from air appeared to exceed the quantity consumed by fish as 4 dead fishes remained in the jar. Accordingly there was found an increasing tendency, amounting to 0.95 cc per litre at the end of the experiment.

Cyprinus, Acidosis Experiment 8.
(Dec 29th, 10.30 am, 1927)

The decrease of CO_2-content found in the preceding experiments may be associated with the diminution of oxygen tension in breathing water. And hence I made here an experiment in which the O_2-content of breathing water was allowed to decrease by the respiration of fishes, and therefore no acid was added to the breathing water.

6 fishes employed were all 5 years in age, measuring 2.985 gms in total weight. One of them was left in tap water for the purpose of control. The dimensions of each fish were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	30.0	8.2	480
B	31.5	8.1	508
C	29.0	8.5	470
D	31.0	9.0	510
E	30.0	8.3	541
F	29.5	8.1	476

Summarizing the following results we might say, in short, that the blood pH responds very sensitively to the change of oxygen tension, diminishing or recovering its value according to the lowering or rising of the oxygen tension. The CO_2-content also responds to the oxygen change in concert with the pH. but its sensitivity is not so sharp as the blood pH.

As regards the condition of fishes during the experiment no change

TABLE 28.

Case No.	Time	Air temp. (C)	Breathing water Temp (C)	pH	O₂ (cc)	Behaviour of fishes	Item	A	B	C	D	E	Mean	F
1	0 m	8°	16.0°	6.5	8.101	—	pH	7.53	7.87	7.70	7.85	7.70	7.74	7.83
						Quiet	CO₂ vol%	40.7	58.4	32.4	39.7	40.7	42.4	42.7
	30	9°	16.0°	6.1	—									
1′	45	10°	16.0°	6.0	2.745	Tended to float	Freq of Resp	70	61	75	68	80	71	26
	1 h	10°	16.0°	5.6	1.460	Floated		57	63	80	55	75	66	28
	2	10°	16.0°	5.6	0.525	Fish E lay		65	63	70	62	76	67	30
2	3	10°			0.525	Somewhat weakened	pH	7.00	7.50	6.85	7.00	7.10	7.10	7.85
	4	10°	16.0°	5.6	0.612		CO₂ vol%	14	55.3	15.9	27.3	22.0	27.0	41.7
							Freq of resp	60	60	70	63	76	66	27
2′	4.30	10°	4.1°	6.5	—	—								
3	5	10°					pH	7.70	7.83	7.53	7.80	7.60	7.69	7.85
	5.30	10°	4 1°	6.5	7 748	Recovered	CO₂ vol%	17.9	37.6	12.7	23.1	27.3	23.7	43.9

	Blood pH (Fish)						CO₂ vol% (Fish)					
Time	A	B	C	D	E	F	A	B	C	D	E	F
0 hr	7.53	7.87	7.70	7.85	7.70	7.83	40.7	58.4	32.4	39.7	40.7	42.7
3	7.00	7.50	6.85	7.00	7.10	7.85	14.7	55 3	15.9	27.3	22.0	41.7
	7.70	7.83	7.53	7.80	7.60	7.85	17 9	37.6	12.7	23.1	27.3	43.9

was observed till after 30 minutes. After 45 minutes the fishes appeared to feel the lack of oxygen, floating up to the water surface. 2 hours afterwards fish E became weakened and lay afloat on the surface. Other fishes also showed marked weakness after 3 hours but they not only recovered the activity as soon as the fresh tap water was supplied but also survived longer.

Cyprinus, Acidosis Experiment 9.
(Dec 31st, 9 40 am., 1927)

Since it was found from the preceding experiment, (Exp 8) that the decrease of O_2 tension in the breathing water and the fall of the pH and CO_2-content of fish blood a further experiment was undertaken with a view to make clearer the aforementioned relation quantitatively. Although the determinations were made on 1 fish, 5 other fishes (which measured 29.0–31.0 cm in body length, 2.785 gms in total weight) were empolyed in order to decrease the O_2 tension of breathing water, and one other fish was used for the purpose of control. Prior to the experiment all fishes were kept in the tap water at 4°C then were transferred to the breathing water at 15.°5 C, but the control fish was kept in the tap water from the beginning of the experiment.

The dimensions of the fishes were as follows.

	Body length (cm)	Body height (cm)	Body weight (gms)
Experimental fish	36.0	9.6	780
Control fish	32.0	8.5	600

TABLE 29.

Case No.	Time	Air temp. (C)	Breathing water Temp. (C)	pH	O_2 (cc)	pH Exp. fish	pH cont-rol fish	CO_2 vol% Exp fish	CO_2 vol% cont-rol fish	Resp freq	Behaviour of fishes
1	0m	10.0°	4.0°	7.25	7.8677	7.50	7.70	42.0	30.9	28	Normal
2	5	10.0°	15.5°	6.40	7.0324					47	Excited
3	10	10.5°	15.5°	6.20	5.9639					60	A little excited
4	15	10.9°	15.5°	6.10	4.8566	7.47		41.0		67	Still
5	20	11.2°	15.3°	6.10	3.4288					70	,,
6	30	11.3°	15.3°	6.00	2.0888					81	Breath regular
7	1 h 30 m	11.3°	15.2°	3.90	0.6216	7.25	7.75	23.8	35.9	60	One gasped
8	1.30	11.5°	15.0°	5.60	0.5245	7.10	7.77	15.9	32.9	57	2 lay down.
9	2 h	11.9°	15.8°	5.60	0.3788	6.95	7.75	14.9	30.9	48	3 lay down.
10	3	12.5°	16.1°	5.60	0.2400	6.80	7.75	13.9	31.9	50	All weakened.

Cyprinus, Acidosis Experiment 10.
(Jan 12th, 10 00 am., 1928)

The preceding two experiments (Exp. 8 and 9) demonstrated that the deficiency of O_2 in the breathing water made the blood pH and CO_2-content decrease.

Therefore I now realized that the change of the pH and CO_2-content observed in the first 8 experiments (Exp. No. 1–8) may or may not be due to the high acidity of water. Because in these experiments also the acidosis may have been due to the O_2 deficiency, and not to the effect of acid which was added to the breathing water.

Therefore I conducted here an experiment in which the pH of breathing water was lowered by the addition of acid, keeping the O_2 content high, thus examining the effects of the two causes separately. In this experiment pure O_2 gas was bubbled through the water with the hope of preventing the decrease of oxygen   The pH of the water was made 3.70 at the start.   The temperature of the water was 8.°7 C at first but it was gradually raised by heating, and made 17.°8 and 20°.0 after 30 minutes and 1 hour respectively.

The 6 fishes used were 5 years in age measuring 3.421 gms in total weight.   The dimensions of each fish were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	29.0	8.3	450
B	30.0	9.0	533
C	30.5	8.2	526
D	31.0	9.0	590
E	29.5	7.6	440
F	34.0	10.7	882

The bleeding was made 2 times·on fish C and 1 time on each of the other fishes. (Table 30).

Cyprinus, Acidosis Experiment 11.
(Jan 13th, 10.00 am., 1928).

The object of the present experiment consisted in producing the acidosis by the low pH of water, eliminating the influence of the O_2 decrease of water, bubbling through the breathing water with the mixture of pure O_2 and air. For this purpose a spiral metal pipe

TABLE 30.

Case No.	Time	Air temp. (C)	Breathing water			Blood		Resp. freq.	Behaviour of fishes	Fish
			Temp. (C)	pH	O_2 (cc)	pH	CO_2 vol%			
1	0m	8.0°	8.7°	3.70	7.654	7.50	37.0	45	Somewhat excited.	F
2	5	8.0°	9.8°	3.75	7.350			50	Quiet	
3	10	8.0°	11.0°	3.80	6.142				„	
4	20	8.0°	14.0°	3.80	4.662	7.47	38.0	60	„	B
5	30	8.0°	17.8°	3.84	3.696	7.48	37.0	67	1 fish lay on the bottom.	C
6	40	8.0°	20.5°	4.00	2.752	7.37	39.0	63	All fishes afloat, a little weakened.	D
7	1h	8.0°	20.2°	4.05	1.679	6.80	20.0	60	2 fishes lay down on the bottom	A
8	2	8.0°	20.2°	4.20	1.219	7.13	20.0	58	3 fishes lay down on the bottom	C
9	3	8.0°	20.2°	4.40	0.872	6.90	18.8	62	4 fishes lay down.	E

having numerous fine pores on its wall, and measuring 1 m in length and 15 mm in diameter was sunk in the water. Connecting one end of this tube with the air pump a stream of fine bubbles was constantly supplied through the water, thereby supplying the O_2 and expelling CO_2 from the water.

3 fishes A, B, and C, were used for the first 3 hours. But it was found after 3 hours that the pH and CO_2-content showed no change when the O_2 tension of water was kept high. So that the experiment was extended to determine whether the fishes would decrease its pH and CO_2-content pronouncedly, if the O_2 tension of breathing water was lowered thereafter. For this purpose three other fishes, D, E, and F, were placed into the jar and thereafter the aeration was ceased.

The determination of the pH and CO_2-content was made 19 times on the first three fishes A, B and C. Among these three fishes A was most throughly studied, 9 determinations being made within 5 hours.

The dimensions of the 6 fishes employed for the present experiment were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	32.3	9.0	560
B	32.0	9.4	572

C	30.3	8.1	430
D	34.0	10.7	882
E	31.0	8.8	538
F	29.5	8.2	450

TABLE   31

Case No.	Time	Air temp. (C)	Breathing water			Blood		Resp. freq.	Behaviour of fishes	Fish
			Temp. (C)	pH	O$_2$ (cc)	pH	CO$_2$ vol%			
1	0m	9.0°	5.0°	3.60	8.192	7.75	43.6	39	A little excited	A
2	5	9.0°	7.5°	3.80	7.529			39	Active.	
3	10	9.0°	6.0°	4.00	7.361			33	Normal, vigorous	
4	15	9.0°	9.6°	4.05					,,	
5	20	9.0°	10.6°	4.10	6.887			74	,,	
6	30	10.0°	12.9°	4.20	6.531	7.77	46.5	60	,,	A
7	40	10.0°	14.0°	4.40	6.311	7.85	42.4	55	,,	B
8	50	10.0°	15.0°	4.50		7.91	41.4	55	,,	C
9	1.00 (h m)	10.0°	15.0°	4.60	6.112	7.82	40.0	55	,,	A
10	1.15	10.0°	15.0°	4.60	6.112	7.85	40.4		,,	B
11	1.30	10.0°	15.0°	4.90	6.112			52	,,	
12	1.40	11.0°	15.0°	5.30		7.90	42.3	55	,,	C
13	2.00	11.0°	15.0°	5.50	6.048	7.80	48.4	52	,,	A
14	2.15	11.0°	15.0°	5.80	5.900				,,	
15	2.30	11.0°	15.0°	6.10		7.85	43.1	67	,,	B
16	2.45	11.0°	14.9°	6.15		7.84	43.9		,,	C
17	3.00	11.0°	14.8°	6.15	5.823	7.80	38.3	55	,,	A
18	3.05	11.0°	14.8°	6.15	5.712			51	Remained still on the bottom	
19	3.10	11.0°	14.9°	6.15	5.386	7.49	30.1	61	,,	B
20	3.15	11.0°	14.9°	6.00	5.019				,,	
21	3.20	11.0°	15.0°	6.00	4.231	7.52	29.1	57	One afloat and gasping.	C
22	3.30	11.0°	15.0°	6.00	3.400	7.80	42.3	71	Some fishes shuke its head.	A
23	4.00	11.0°	15.0°	6.00	1.575	7.65	36.2	67	Afloat, agonized.	A
24	4.20	11.0°	15.0°	5.90					,,	
25	4.30	11.0°	15.0°	5.80	0.914	7.20	27.1	58	Fish B much weakened.	A
26	4.40	11.0°	15.0°	5.80		7.25	27.1	57	Fish C weakened.	B
27	4.50	11.0°	15.0°	5.80		6.90	19.9		All fishes weakened.	C
28	5.00	11.0°	15.0°	5.80	0.830	7.00	23.7	60	,,	A

The present experiment showed that the blood pH of fishes does not decrease in spite of the low pH of breathing water, provided the O_2 was fully supplied. But when the O_2 began to decrease the blood pH also immediately showed a decreasing tendency. Therefore it can be concluded now that the O_2 decrease was a sole cause of the change of blood pH in this case, and that the low pH of the breathing water had nothing to do with change of blood pH.

During aeration the fishes showed no weakness until 3 hours after the beginning, and they lived actively without any indication of the effect of low pH water.

But following a marked depression of O_2 tension which occurred towards the end of the 3rd hour, the fishes sensitively reacted to this adverse condition. 20 minutes after the aeration was ceased one of them floated on the water surface making a gasp. 1 hour afterwards all the fishes came to the water surface and breathed the air together with water. From 1 and a half hours afterwards all the fishes were somewhat weakened. At the end of the experiment fish C lay afloat on the surface, but it recovered the activity together with the other fishes as soon as fresh tap water was supplied.

<div align="center">

Cyprinus, Acidosis Experiment 12

(Jan. 15th, 9.00 am., 1928).

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Since the blood pH of fish can never be lowered by the low pH of water as long as the O_2 is kept in its usual quantity, the duration of the preceding experiment may be considered too short (3 hours). Therefore the duration was lengthened in the present experiment with an intention to verify whether the long exposure of fishes to the high acidity alters the blood pH or not.

The measurements of the fishes were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	31.0	9.0	546
B	32.0	9.7	612

As will be seen from the following table an appreciable decrease in blood pH was found in one hand, but on the other hand a prominent decrease of O_2 in breathing water was observed. Therefore the lower-

TABLE 32.

Case No.	Time	Air temp. (C)	Breathing water			Blood		Resp. freq.	Behaviour of fishes	Fish
			Temp. (C)	pH	O₂ (cc)	pH	CO₂ vol%			
1	h m 4.30	10.5°	12.5°	4.30	5.590	7.70	46.5	60	Normal, active	A
	4.40	10.5°	12.5°	4.30	–	7.70	38.4	60	,,	B
2	6.00	11.3°	16.5°	4.10	4.902	7.68	39.4	60	,,	A
	6.10	11.3°	16.5°	4.10	—	7.65	35.2	55	,,	B
3	7.00	11.3°	14.2°	4.20	4.892	7.45	39.3	48	,,	A
	7.10	11.3°	14.2°	4.20	—	7.55	37.3	40	,,	B

ing of blood pH in this case may probably be due to the O₂ decrease in breathing water instead of being due to the low pH of breathing water. If the low pH of water also decreases the blood pH, a further distinct fall of pH had to be detected in these cases. Because the

TABLE 33.

Case No.	Time	Air temp. (C)	Breathing water		O₂ (cc)	HCl (cc) added
			pH	Temp. (C)		
1	0m	6.0	3.70	3.5	8.121	55
2	5	6.0	3.75	6.2	7.835	0
3	10	6.5	3.80	8.0	6.988	0
4	15	6.5	3.85	8.5	6.967	0
5	20	7.5	3.90	8.5	6.617	5
6	30	·8.0	3.80	10.0	5.834	5
7	45	8.0	3.90	12.5	—	5
8	h m 1.00	8.0	4.00	12.7	—	5
9	1.15	8.3	4.05	12.8	—	5
10	1.30	8.3	4.20	12.8	5.833	10
11	1.45	8.3	4.10	12.6	—	5
12	2.00	8.3	4.20	12.6	5.823	15
13	2.15	8.3	4.00	13.2	—	5
14	2.30	8.0	4.00	13.2	—	10
15	2.45	8.0	4.00	13.8	—	10
16	3.00	8.0	3.95	13.9	·5.664	10
17	4.00	10.0	4.60	17.0	5.590	20
18	4.30	10.0	4.30	16.5	—	10
19	5.00	10.5	4.00	16.5	4.934	10
20	5.30	10.5	4.20	16.5	—	20
21	6.00	11.3	4.10	15.5	4.902	10
22	6.30	11.3	4.10	15.0	—	10
23	7.00	11.3	4.20	14.2	4.892	0

O_2 decrease of water in the above case would also have decreased the blood pH. Moreover, we can expect from the preceding experiment that such a decrease in O_2 as found here would produce an appreciable fall of blood pH. Therefore we may conclude that such a degree of low pH of the breathing water used in this experiment might not lower the blood pH to any appreciable extent. The Table 33 shows the change of conditions during the experiment.

B) ALKALOSIS.

Cyprinus, Alkalosis Experiment I.
(Jan. 17th, 7.00 a.m., 1927).

In the present experiment, about 17 cc of n/5 NaOH solution was added to 20 litres of tap water which showed the pH 7.30 and the

TABLE 34.

Case No.	Time	Air temp. (C)	Breathing water			Blood		Resp. freq.	Behaviour of fishes
			Temp. (C)	pH	O_2 (cc)	pH	CO_2 vol%		
1	0m	7.0	8.3	9.00	7.379	7.64	36.3	—	
2	5	7.7	7.2	9.00				55	Startled, excited.
3	10	7.7	7.2	8.95	6.991			55	,,
4	15	7.7	7.5	8.95				55	,,
5	20	7.7	7.8	8.90	6.592			58	Became calm.
6	30	7.7	8.2	8.80	5.606			65	,,
7	1.00 h m	9.0	8.8	8.95	4.451	7.63	35.3	68	,,
8	1.30	11.0	11.0	9.15	2.971			72	,,
9	2.00	10.0	11.0	9.15				73	Still.
10	2.30	10.0	11.2	9.15				70	,,
11	3.00	10.0	11.7	9.20	1.711	7.65	30.1	57	,,
12	3.30	10.0	14.0	9.15		7.65	28.2	70	,,
13	4.00	10.0	16.5	9.10		7.70	23.7	77	Breathing actively.
14	4.30	10.0	16.7	9.05				71	,,
15	5.00	10.0	16.5	9.05		7.75	18.8	67	,,
16	5.30	9.0	16.0	9.12				61	,,
17	6.00	9.0	16.0	9.30		7.75	13.7	59	A little weakened.
18	15.00	8.0	8.8	9.30		8.00	19.8	33	,,
19	39.00	8.0	12.0	9.15		7.43	4.5	40	Died soon after.

pH was raised to 9.00. As soon as the fishes were put in the n/5
NaOH solution was dropped in by an aspirating dropper to keep the
pH constantly high. Next, the alkali solution was added so as to
maintain the pH at 9.00, and about 6 to 20 drops per minute were
needed for that purpose.

The water was renewed after the estimation of the 6th hour and
the alkalinity was regulated to pH 9.0 by adding the aforesaid alkali
solution.

As it was necessary to bleed the same fish several times, a some-
what large individual (length 32 cm, height 11. cm, weight 723 gms)
was chosen in this experiment.

The fish material used in the present investigation showed hyper
activity, and behaved roughly in the experiment tank until one and
a half hours later. Thereafter they became quiet and breathed nor-
mally until 3 to 4 hours. Meanwhile it appeared very indolent showing
little debility after 6 hours. Though they remained very quitely on
the bottom of the experimental tank until 39 hours later, it could not
recover the vigour by the renewal of water and died thereafter.

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Cyprinus, Alkalosis Experiment 2.

(March 17th, 9 00 am., 1927)

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According to the former experiment (Exp. 1) the pH of the blood
shows slight elevation when the alkalinity of breathing water is kept
higher than pH 9.00 provided the water temperature is higher than
10°C. But we observed that the increase of pH is somewhat less
than we expected, probably because of the decrease of the O_2 content
of the water. In addition the CO_2-content of the blood was also
markedly decreased for the same reason. In the present experiment,
however, I tried to prevent the lowering of the pH and CO_2-content
by supplying oxygen or by renewing the whole breathing water.

The dimensions of the fishes were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	33.0	8.3	557
B	30.1	8.6	556

TABLE 35.

Case No.	Time	Air temp. (C)	Breathing water Temp. (C)	pH	O₂ (cc)	Blood pH A	pH B	CO₂ vol% A	CO₂ vol% B	Resp. freq. A	Resp. freq. B	Behaviour of fishes
1	0m	8.0°	13.2°	9.20	7.020	7.98	7.97	49.9	47.5	48	35	Still.
2	5	10.0°	13.2°	7.80	6.594							,,
3	10	10.0°	13.5°	8.70	6.898							Fish B excited.
4	15	10.2°	13.5°	8.50	6.765							
5	20	10.5°	13.5°	8.95	6.765							Fish B shuke its head convulsively.
6	30	10.5°	13.5°	8.90	6.807	7.80	7.80	44.4	38.0	67		Fish A excited.
7	h m 1.00	10.5°	13.3°	9.10	6.625	7.80			41.6	60	50	
8	1.30	12.0°	14.0°	9.30	6.520	7.80			41.2	50		
9	2.00	13.0°	14.6°	9.40	6.233	7.70	7.80	40.6	28.0	55	60	
10	2.30	12.0°	15.2°	9.40	6.009					55	60	Quiet.
11	3.00	13.0°	16.6°	9.40	6.094	7.55	7.55	38.3	25.0	61	58	
12	3.30	13.0°	15.6°	9.40	5.988	7.50	7.65	37.3	21.0	60	55	A little weakened.
13	4.00	12.7°	14.3°	8.80	6.307	7.65	7.60	36.1	25.1	51	55	
14	4.30	14.0°	12.5°	9.40	6.775					47	52	
15	5.00	14.0°	14.8°	9.20	6.360	7.67	7.65	37.0	21.0	55	49	
16	5.30	14.2°	15.7°	9.40	6.222					58	52	
17	6.00	15.5°	16.2°	9.20	5.690	7.69	7.68	37.0	21.0	55	64	
18	6.30	16.5°	17.5°	9.00	5.850					60	67	
19	7.00	15.5°	17.7°	8.70	5.829	7.78	7.70	34.0	19.0	60	64	
20	24.00	8.0°	7.0°	8.90	6.383	8.15	8.05	21.0	12.0	32	26	Recovered.

Cyprinus, Alkalosis Experiment 3.
(March 20th, 9.30 am., 1927).

The preceding two experiments (Exp. 1. 2) showed that the blood pH markedly increases after 15 hours, but the increase within several hours is very indistinct. In the present experiment, therefore, the pH of the breathing water was kept much higher than that of the former experiments and it was attempted to raise the blood pH distinctly within a few hours. Since the higher water temperature and the successive frequent bleeding from an individual quicken the

exhaustion of fishes the temperature was held under 12°C and the bleeding was done less frequently.

The fishes employed were four years of ago, having the following dimensions. The bleeding was accomplished six times with each fish, i. e., at the beginning, after 4 hours, 5 hours, 7 hours, 24 hours, and after 72 hours, determining the pH value and the CO_2-content each time.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	29.5	8.4	457
B	29.8	9.3	544

TABLE 36.

Case No.	Time	Air temp. (C)	Breathing water			Blood					Resp freq		Behaviour of fishes
			Temp. (C)	pH	O_2 (cc)	pH		CO_2 vol%			A	B	
						A	B	A	B				
1	0m	10.8	7.6	9.10	7.968	8.00	8.20	51.6	49.2		43	39	Quiet.
2	30	11.0	8.3	9.30							40	35	
3	h m 1.00	11.0	9.0	9.80							48	46	Increased reflex of resp.
4	1.30	12.5	9.6	9.80							50	48	
5	2.00	12.0	10.3	9.80							52	55	
6	2.30	12.5	10.6	9.80							46	58	
7	3.00	12.0	10.7	9.80							60	60	Breath regular.
8	3.30	11.5	11.0	9.80							60	51	
9	4.00	11.0	11.5	9.80		8.25	8.30	48.4	35.0		56	51	Tended to float.
10	4.30	11.0	11.6	9.80							60	55	
11	5.00	12.0	11.8	9.80		8.30	8.35	48.4	31.0		55	52	
12	5.30	13.0	12.0	9.80							60	49	Floated on the surface.
13	6.00	13.0	12.0	9.80							58	51	
14	6.30	12.0	11.8	9.80							55	46	
15	7.00	11.2	10.8	9.80	0.933	8.30	8.35	34.8	20.4		57	40	A little weakened.
16	24.00	8.0	3.6	6.90		8.35	8.35	59.6	55.0		—	—	Recovered.
17	72.00	9.0	4.6	6.70	7.886	8.30	8.20	66.4	55.0		25	22	Vigorous.

Looking through the change of blood pH in both fishes one will note two remarkable facts. First, in the present experiment the in-

crease of blood pH was so rapid that such a quick increase was never seen in the former case, namely both fishes showed a distinct increase within 4 hours and reached the highest value after 5 hours. This is a great difference when compared with the former experiment in which 15 hours elapsed before a distinct increase was seen. Second, after 7 hours the blood pH kept a very high value, yet despite the pH of breathing water returned to the normal value by this time. This is also a remarkable difference from the first experiment of alkalosis, and reserved a question for further investigation.

Cyprinus, Alkalosis Experiment 4.
(March 22nd, 9.30 am., 1927.)

By the Alkalosis Experiment 3 we have found that the blood pH can be raised within 4 hours by increasing the pH of breathing water up to 9.80, and have noticed moreover that the change of the pH and CO_2-content show a very interesting relation. In the present experiment, therefore, a similar method was repeated, to confirm the result of the Alkalosis Experiment 3 on the one hand, and to determine the maximum pH attainable on the other. The measurements of the fishes used were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	29.3	9.1	510
B	30.0	8.3	480

TABLE 37.

Case No.	Time	Air temp. (C)	Breathing water Temp. (C)	pH	O$_2$ (cc)	Blood pH A	Blood pH B	Blood CO$_2$ vol% A	Blood CO$_2$ vol% B	Resp. freq. A	Resp. freq. B	Behaviour of fishes
1	0m	10.0°	7.0°	9.10	7.918	7.95	7.57	50.6	48.3	37	39	Somewhat excited.
2	10	12.0°	7.5°	9.20	7.831							
3	20	12.8°	7.7°	9.20	7.398							
4	30	13.0°	7.8°	9.40	7.105							

(Continued)

Case No.	Time	Air temp. (C)	Breathing water			Blood				Resp. freq.		Behaviour of fishes
			Temp. (C)	pH	O_2 (cc)	pH		CO_2 vol%				
						A	B	A	B	A	B	
	h m											
5	1.00	13.0°	8.8°	9.80	6.042					43	48	Quiet.
6	1.30	12.2°	9.6°	9.80	5.070					39	55	
7	2.00	11.7°	10.5°	9.80	3.973					40	50	
8	2.30	12.3°	10.8°	9.80	3.563					43	57	
9	3.00	12.5°	11.3°	9.80	2.766	8.25	7.85	43.2	36.2	48	50	A little excited.
10	3.30	12 7°	11.7°	9.80	2.730					58	55	
11	4.00	12.5°	12.0°	9.80	2.278	8.50	7.98	36.5	36.1	44	55	
12	4.30	12.5°	12.2°	9.80	1.952					46	60	
13	5.00	12.5°	12.1°	9.80	1.627	8.35	9.25	32.1	36.2	40	49	Floated on the surface shuke their heads convulsively.
14	5.30	11.5°	11.9°	9.80	1.493					33	36	
15	6.00	11.0°	12.4°	9.80	1.410					33	34	A little weakend.
16	6.30	10.7°	12.4°	9.80	1.378					37	42	
17	7.00	11.0°	12.5°	6.70	1.356	8.33	7.98	26.1	5.9	33	33	Quiet.
18	24.00	9.0°	4.6°	6.70	7.050	8.30	8.27	41.6	46.9	24	22	Recovered.
19	48.00	9.3°	3.0°	6.70	7.624	8.25	8.22	63.3	59.1	25	26	Vigorous.

Cyprinus, Alkalosis Experiment 5.
(March 23rd, 1 00 am, 1927)

As it was demonstrated by the preceding two experiments (Exp. 3. 4) that the pH of the blood can apparently be raised within 4 hours when the alkalinity of the water is extraordinarily increased, a further experiment was planned to determine the mode of the rise of blood pH within 4 hours, and moreover to try to raise the blood pH as high as possible by this method.

The dimensions of the fishes used were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	30.3	9.4	560
B	30.5	9.0	492

TABLE 38.

Case No.	Time	Air temp. (C)	Breathing water			Blood				Resp. freq.		Behaviour of fishes
			Temp. (C)	pH	O₂ (cc)	pH		CO₂ vol%				
						A	B	A	B	A	B	
1	0m	9.3	5.0	9.10	7.910	7.85	7.95	39.7	43.8	—	—	Quiet.
2	10	9.7	5.5	9.20	7.907					39	40	Excited.
3	20	11.0	5.7	9.40	7.519					—	—	
4	30 h m	11.7	6.0	9.60	7.209	7.63	7.67	36.2	31.8	45	40	
5	1.00	12.0	7.2	9.80	6.182	7.67	7.76	35.2	40.3	44	36	Water contaminated
6	1.30	12.5	8.7	9.80	5.230					48	41	Water contaminated
7	2.00	11.7	9.8	9.80	4.246	7.95	7.83	35.2	28.0	59	46	
8	2.30	11.7	11.2	9.80	3.439					55	50	
9	3.00	11.5	11.5	9.80	2.424	8.03	7.93	28.3	26.0	56	55	Active
10	3.30	12.0	11.8	9.80	2.103					59	52	
11	4.00	11.4	12.0	9.80	1.574	8.23	8.15	27.6	25.0	55	51	Quiet
12	4.30	11.5	12.0	9.80	1.315					48	49	
13	5.00	12.0	12.0	9.80	1.181	8.23	8.25	25.0	23.0	48	50	Somewhat weakened
14	5.33	13.0	12.0	9.80	1.139					48	48	
15	6.00	13.0	11.8	9.80	1.159	8.23	8.22	18.9	17.3	50	50	
16	6.30	13.0	11.9	9.80	1.121					48	50	Fish A much weakened
17	7.00	12.7	11.9	9.80	1.107	8.22	8.19	16.9	15.9	46	50	
18	24.00	9.5	3.0	6.70	7.104	8.20	8.13	48.9	45.0	26	25	Recovered.
19	48.00	9.7	3.5	6.70	7.345	8.05	8.00	46.5	47.5	24	23	Normal.

II. EXPERIMENT ON *LEUCISCUS*.

A) ACIDOSIS.

Leuciscus, Acidosis Experiment 1.

(May 9th, 9.00 am., 1927).

The present experiment was conducted in order determine whether *Leuciscus* behaves like *Cyprinus*, producing the acidosis by the decrease of O₂ tension in breathing water. The experimental procedure was the same as that of the experiment on *Cyprinus*. Three fishes were

subjected to respire in 20 litres of sea water in which the O_2 was allowed to decrease by the respiration of fishes.

The sea water used showed its pH to be 8.15 and the specific gravity to be 1.0227 (10.°4).

The measurements of the fishes used were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	36.2	7.5	500
B	33.3	6.9	428
C	35.4	8.0	528

TABLE 39.

Case No.	Time	Air temp. (C)	Temp (C)	pH	O_2 (cc)	Blood pH A	B	C	CO_2 vol% A	B	C	Resp. freq A	B	C	Behaviour of fishes
1	0 m	13.8	10.4	8.15	6.125	7.50			22.7			86	87	75	Active.
2	5	13.9	10.4		5.637		7.25			17.8					Swam actively.
3	10	13.9	10.4	7.97	5.169			7.30			16.8				
4	15	14.0	10.5												Still.
5	20	14.0	10.5	7.80	3.984							91	97	80	All fishes still.
6	30	14.0	10.5		3.518										
7	40	14.0	10.6	7.50								94	100	60	
8	1.00	14.2	10.7	7.30	1.614	7.35	7.05	7.25	16.8	17.8	15.8	94	103	63	Fish C weakened.
9	1.30	15.0	11.0	7.20	0.956							92	79	48	C afloat, agonized.
10	2.00	15.0	11.1	7.10	0.797	7.30	6.90	7.00	13.9	12.8	14.1	78	86	38	B lay down.
11	2.30	15.0	11.2	7.10	0.867							60			B, C swam convulsively.
12	3.00	15.0	11.4		0.677	7.25			13.9			60			A somewhat weakened.
13	3.30	15.7	11.5	7.00	0.657							52			A much weakened.
14	4.00	16.0	11.7	7.00	0.647	7.10			13.1			52			B, C died.
15	4.30	16.0	11.7	7.00	0.687							51			A lay down.
16	5.00	17.0	12.0	7.00	0.689	7.00			12.3			51			A died.

In this experiment fishes B and C which had relatively low initial value pH began to diminish below 7.00 as early as within 2 hours, and died shortly later. While fish A which showed the highest in

its initial pH value maintained the normal value as long as 3 hours and died after 5 hours when the pH decreased below 7.00. These facts seem to suggest that the fishes which have a rich alkali reserve are more resistant than those which have but a little alkali reserve.

Leuciscus, Acidosis Experiment 2.
(May 10th, 9.00 am., 1927).

The former experiment showed us that *Leuciscus* also decreases its blood pH and CO_2-content by O_2 deficiency like *Cyprinus*. But in the former experiment whether such changes occur so rapidly as within 1 hour could not determined as the blood test was not made within 1 hour. Therefore, in this experiment the changes which occur within 1 hour have been determined.

Each of four fishes which were employed for this experiment was bled before being placed into the experimental jar, and thus its normal pH and CO_2-content were determined.

The first bleeding was made on fishes A and B 20 minutes after placing them in the experimental jar, and on fishes C and D after 40 minutes. Thereafter the two lots of fishes were bled alternately at an interval of 30 minutes. Besides these four fishes another fish E was used for the purpose of control, keeping it in running tap water.

The breathing sea water showed its pH and specific gravity to be 8.16 and 1.0247 (11°) respectively. The renewal of water was not made till the experiment was finished.

The measurements of fishes used were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	36.0	8.0	537
B	36.0	7.5	472
C	35.0	7.5	497
D	36.0	8.7	624
E	34.5	7.0	470

From the result of the present experiment I have found a tendency which seems to suggest to us that the magnitude of pH and CO_2-content have some special bearing upon the life duration as can be seen in the Table 41.

TABLE 40.

Case No.	Time	Air temp. (°C)	Breathing water Temp. (°C)	pH	O₂ (cc)	Blood pH A	B	C	D	E	CO₂ vol% A	B	C	D	E	Resp. freq. A	B	C	D	E	Behaviour of fishes
1	0m	16.0°	11.0°	8.16	6.106	7.35	7.36	7.45	7.39		18.6	13.7	16.7	19.6	16.7	100	100	86	103	107	Highly excited.
2	5	16.0°	11.1°	8.04	4.811											86	100	86	115	86	Became quiet.
3	10	16.0°	11.2°	7.90	3.904											103	107	100	107	73	Breathed excitedly.
4	20	16.1°	11.2°	7.65	1.848	7.26		7.48								86	86	102	100	71	Felt O₂ deficiency.
5	30	16.3°	11.3°	7.40	0.747	7.25	7.50				15.7	16.7				75	68	77	82	67	All agonized. B floated.
6	40	17.0°	11.5°	7.28	0.667	7.30	7.00				7.29	22.5	18.6			56	56	56			C floated upside down.
7	1.00 h m	17.0°	11.7°	7.30	0.608	7.00	.25				17.6								40		A breathed intermittently.
8	1.20	17.0°	11.8°	7.10	0.618	7.00	6.90				18.6	17.6	15.7	17.5					79		B and C dying.
9	2.00	17.7°	12.2°	7.10	0.697	7.16					18.9								65		A nearly died.
10	2.30	18.2°	12.2°	7.00	0.887	7.00	7.30				12.0	16.7							67		D nearly died.

TABLE 41.

Fish	pH	CO$_2$ vol%	Life duration
			h m
D	7.45	19.6	2.30
A	7.35	18.6	1.30
C	7.25	16.7	1.00
B	7.20	13.7	0.40

Leuciscus, Acidosis Experiment 3.
(May 12th, 9.00 am., 1927).

The present experiment was made with the hope to verify the results of the former two experiments (Exp. 1. 2). The method of experiment was almost the same as in the former cases.

Among three fishes employed, fish A was injured as it acted violently when blood was collected and died 5 minutes later. Therefore subsequent bleeding was made on the two remaining fishes B and C, bleeding 9 times in all. The pH of the sea water was 8.20 and the specific gravity was 1.0245 (10°).

The measurements of the fishes used were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	38.0	8.0	614
B	41.0	9.5	890
C	36.5	7.9	520

Like the preceding experiments it was also found in the present case that the individuals which have the highest pH survived longest (Table 42).

Leuciscus, Acidosis Experiment 4.
(May 15th, 9.30 am., 1927).

The present experiment was also made with the object of obtaining data to verify the results of the preceding three experiments (Exp. 1–3). And hence the method employed was quite the same as in the former experiment.

Three fishes A, B, and C were employed. The blood collection was made 4 times on fishes A and C, and 3 times on fish B. The

TABLE 42.

Case No.	Time	Air temp. (°C)	Breathing water Temp. (°C)	pH	O₂ (cc)	Blood pH A	B	C	Mean	Blood CO₂ vol% A	B	C	Mean	Resp. freq. A	B	C	Behaviour of fishes
1	0m	13.0°	10.5°	8.30	6.656	7.28	7.07		7.40	19.8	17.0	21.0	19.3	88	79	84	Active.
2	5	13.1°	10.5°	8.15	5.994										80	84	Quiet, A died.
3	10	13.2°	10.5°	8.12	5.102										77	89	B and C gasping.
4	20	13.5°	10.5°	7.90	3.789										75	91	,,
5	30	13.9°	10.6°	7.30	3.400	7.10	7.20		7.15	17.0		18.5	17.8		96	100	B, C increase resp. freq.
6	40 h m	13.9°	10.7°	7.25	1.854										91	91	B and C agonized.
7	1.00	13.9°	10.7°	7.20	0.924		7.20	7.20	7.20		17.0	—	17.0		75	75	B much weakened.
8	1.30	13.9°	10.5°	7.10	0.832		7.05	7.03	7.04		15.0	19.0	17.0		68	50	B, C lay upside down.
9	2.00	13.4°	10.8°	7.10	0.875		7.00	7.00	7.00								B, C died.

TABLE 43.

Case No.	Time	Air temp. (C)	Breathing water Temp. (C)	Breathing water pH	Breathing water O₂ (cc)	Blood pH A	Blood pH B	Blood pH C	Blood pH Mean	Blood CO₂ vol% A	Blood CO₂ vol% B	Blood CO₂ vol% C	Blood CO₂ vol% Mean	Resp. freq. A	Resp. freq. B	Resp. freq. C	Behaviour of fishes
1	0m	14.4°	10.8°	8.18	6.736	7.30	7.18	7.20	7.23	15.8	14.1	15.1	15.0	86	79	84	Swam actively
2	5	14.4°	11.0°	8.13	5.215									86	88	83	Still
3	10	14.5°	11.1°	8.00	3.872									88	94	81	Respired deeply.
4	20	14.5°	11.1°	7.71	2.965									90	94	86	"
5	30	14.5°	11.1°	7.55	1.894	7.33	7.16	7.20	7.22	13.1	13.1	14.1	13.4	94	98	95	"
6	40 h m	14.5°	11.2°	7.35	0.912										67	96	Fish B agonised.
7	1.00	14.4°	11.5°	7.25	0.571	7.03	7.00	7.13	7.05	13.0	12.0	12.1	12.3	55	61	73	B lay upside down; died soon after.
8	1.30	14.5°	11.5°	7.20	0.561	6.90						12.0	12.0	50		55	A lay upside down, died soon after.
9	2.00	14.7°	11.4°	7.15	0.712			6.98	6.98			11.0	11.0				C breathed intermittently.
10	2.30	15.0°	11.5°	7.10	0.594			6.90	6.90			11.0	11.0			60	C died.

temperature of the water was kept a little higher than in the three former experiments. The pH of the sea water was 8.18 and the specific gravity was 1.0236 (10."3).

The measurements of the fishes used were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	38.5	8.7	613
B	36.7	7.2	459
C	37.0	7.3	501

(Table 43).

Leuciscus, Acidosis Experiment 5.
(May 17th, 9.30 am, 1927)

All experiments hitherto conducted on *Leuciscus* were carried out by using the sea water for the breathing of fishes.

But as this fish is anadromous in habit it can live in fresh water as well as in sea water. Therefore an experiment was attempted using fresh water instead of sea water to find whether any difference may be noticed. As the fresh water has low pH and high O_2 content in comparison with the sea water some difference can be anticipated with regard to the change of the blood pH and CO_2-content.

The experiment was carried out in a manner similar to the other experiments. The pH of the fresh water was 7.50 in natural condition. The fishes employed were caught in the open sea and were kept for 89 hours in fresh water to accustom them to the altered condition. One of the fishes was killed accidentally in an occasion of bleeding, and hence the determination was made but one time on this fish. But as regards the other fishes 6 to 8 determinations were made.

The measurements of the fishes were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	34.5	8.0	516
B	36.0	8.0	493
C	35.4	7.2	470

It is worthy of noting that in fresh water the decrease of O_2 is slower than in sea water. The O_2 content of fresh water never decreased below 1.3 cc per litre while in the sea water it always decreased below 1.0 cc per litre. Accordingly the activity of fishes

TABLE 44.

Case No.	Time	Air temp. (C)	Breathing water Temp (C)	Breathing water pH	Breathing water O₂ (cc)	Blood pH A	Blood pH B	Blood pH C	Blood pH Mean	CO₂ vol% A	CO₂ vol% B	CO₂ vol% C	CO₂ vol% Mean	Resp. freq. A	Resp. freq. B	Resp. freq. C	Behaviour of fishes
1	0m	14.4°	11.0°	7.50	6.696	7.06	7.13	7.83	7.17	20.7	16.7	22.7	20.4	100		81	Much excited.
2	5	15.5°	11.5°	7.00	6.096										68	91	Quiet.
3	10	15.0°	11.5°	6.60	5.302										68	86	Startled.
4	20	15.0°	11.5°	6.40	4.491										97	91	
5	30	15.0°	11.5°	6.20	3.886		7.50	7.35	7.42	25.8	25.8	25.8	25.8		83	86	Respired deeply.
6	40	15.5°	11.5°	6.20	2.957										97	85	
7	1.00	16.0°	11.5°	6.10	2.06		7.33	7.42	7.38	24.0	21.0	22.5		91	86	
8	1.30	16.0°	11.7°	6.00	1.614		7.20	7.20	7.20	15.3	17.1	16.2		77	54	Floated, a little weakened.
9	2.00	16.7°	11.9°	5.80	1.418		7.00	7.05	7.03	14.1	15.2	29.2		07	50	C sometimes lay down
10	2.30	17.0°	12.1°	5.80	1.302		6.82	6.90	6.96	14.1	15.7	14.9		58	48	C lay down.
11	3.00	17.0°	12.3°	5.80	1.654		6.70		6.70	15.7				58		B weakened, lay down
12	3.30	17.5°	12.4°	5.80	1.87									58		
13	4.10	18.0°	12.6°	5.80	1.604		6.75		6.76	16.7		16.7				B respired intermittently.

is also affected by this difference. In sea water, for instance, the fishes began to gasp as early as 10 minutes after the start and manifested apparent agony after 30 minutes, while in the fresh water they showed no debility until 1 hour later.

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Leuciscus, Acidosis Experiment 6.

(May 19th, 8 40 am, 1927).

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A comparison of the previous experiment with the other experiments seems to suggest that the blood pH and CO_2-content of *Leuciscus* show no particular difference according whether the fishes were submitted to respire in fresh water or in sea water. But as regards the other relations such as the rate of the decrease of O_2 or the degree of weakness of the fishes some appreciable distinctions are seen to exist. In order to verify these results a further experiment was carried out after same manner as that of the former experiment.

To adapt the fishes to the fresh water they were kept for 44 hours in the fresh water. The bleeding was made 6 times on each fish within 2 and a half hours. 4 fishes were used and one of them D, was left in running tap water for the purpose of control.

The measurements of the fishes used were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	35.1	7.5	475
B	33.1	7.1	405
C	35.5	7.9	547
D	33.2	7.4	502

The change of the pH and CO_2-content in this experiment showed no difference from the other experiments which were conducted employing sea water in place of fresh water.

As was observed in the former experiments the weakness of fishes during the experiment in fresh water appeared undoubtedly slow in contrast with that observed in sea water. The fishes showed no change in their activity until 40 minutes later and they first manifested some debility 1 hour later.

From the results of the present and the former experiments I am tended to concluded that *Leuciscus* shows no particular change in its blood pH and CO_2-content according as the breathing water is sea

TABLE 45.

Case No.	Time	Air temp (C)	Breathing water Temp (C)	pH	O_2 (cc)	Blood pH A	B	C	Mean	D	Blood CO_2 vol% A	B	C	Mean	Resp freq A	B	C	Behaviour of fishes
1	0m	16.5°	12.2°	7.40	6.772	7.07	7 02	7.03	7.04	7.05	15.8	15.8	16.8	16.1	103	111	103	Much excited.
2	5	17.0°	12.2°	6.80	5.777										97	94	94	Became quiet.
3	10	17.0°	12.3°	6.40	4.389										100	97	100	Still.
4	20	17.0°	12.4°	6.25	3.531	7.27	7.22	7.25	7.25		18.7	17 7	15.8	17.4	103	100	100	"
5	30	17.0°	12.5°	6.10	1.968					7.15					111	107	100	Breath regular.
6	40	17.5°	12.7°	6.10	1.466										88	106	98	"
7	1.00	17.4°	12.8°	5.80	0.674	7.18	7.07	7.08	7.11		18.2	18.7	17.7	18.2	86	91	75	Fish A floated C floundered.
8	1.30	17.4°	13.0°	5.80	0.535	6.98	6.98	7.00	6.98	7.05	10.9	11.9	13.8	12.3	70	69	55	A and B lay down.
9	2.00	17.5°	13.4°	5.80	0.888	6.90	6.83	6.86	6.86	7.05	10.9	7.5	11.9	10.1	64	55	40	A and C much weakened.
10	2.30	17.7°	13.5°	5.80	0.899	6.90	6.83	6.70	6.80	7.05	9.0	8.0	7.5	8.2			56	C died, and B breathed intermittently.

water or fresh water. But it was sure, as just mentioned, that the faintness of fishes was manifested apparenty later in fresh water than in sea water.

Leuciscus, Acidosis Experiment 7.
(May 20th, 9 00 am., 1927)

Five experiments hitherto conducted have been made with a view to determine the effect of O_2 deficiency upon the blood pH and CO_2-content. The present experiment was, however, planned with a hope of learning whether the low pH of breathing water affects the pH and CO_2-content of the blood. In *Cyprinus* this relation was already studied and it was found that the high acidity of water never changes the blood pH or CO_2-content. Accordingly the aim of the present experiment consisted in finding the difference in this relation due to the species of fishes.

In order to suit the above mentioned object, the pH of the breathing water was made very low by adding an amount of n/10 HCl solution. In the case of the experiment on *Cyprinus* about 80 cc of n/10 HCl solution was sufficient to make the pH of 20 litres of fresh water 3.90. While in the present experiment in which the sea water (pH 8.20) was used about 640 cc of the n/10 HCl was needed for the same volume of water to lower its pH to 3.40 Though the specific gravity of water indicated 1.0246 (12°.7) at first, it decreased to 1.0241 (12.7) as the HCl solution was added.

The bleeding was made at intervals of 30 minutes. The blood determination was accomplished 3, 4, and 5 times upon fishes A, B, and C respectively. The measurements of the fishes were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	34.9	7.5	531
B	38.4	7.3	549
C	35.8	7.1	'485

Comparing the manner of the pH fall in this experiment with those of the Exps. 1-6 an apparent distinction can be found. In all the experiments hitherto conducted on *Leuciscus* the blood pH always showed a rise within 30 minutes. While in the present experiment,

TABLE 46.

Case No.	Time	Air temp. (C)	Breathing water Temp. (C)	pH	O₂ (cc)	Blood pH A	B	C	Mean	Blood CO₂ vol% A	B	C	Mean	Resp freq A	B	C	Behaviour of fishes
1	6m	16.8°	12.6°	3.40	5.692	7.22	7.10	7.22	7.18	11.8	16.2	17.1	14.7	114	86	102	Vigourous.
2	5	17.6°	12.8°	3.40	5.032									88	79	100	Quiet.
3	10	17.6°	12.8°	3.46	4.638									94	88	96	Breath regular.
4	20	17.6°	12.9°	2.50	3.856									79	73	94	Remained still on the bottom
5	20	17.6°	12.9°	2.50	3.007	6.85	6.80	6.82		22.1			18.1	88	67	94	
6	40	17.2°	13.0°	2.57	2.622												A little weakened.
7	1.00 h.m	17.5°	13.1°	3.57	2.968	6.80	6.70	6.72	6.74	15.2	22.1	17.1	18.1	55	40	78	All lay down. Fish A died.
8	1.20	17.5°	13.2°	3.57	2.052	6.70	6.70	6.70	6.70	15.2	22.1	17.1	19.6			45	B and C breathed faintly.
9	2.00	17.6°	13.4°	3.57	1.947	6.70	6.70	6.70	6.70	22.1	22.1		22.1				B died, C ceased breathing.

on the contrary, the blood pH showed a prominent fall by the same time. On the other hand the O_2 decrease which was hitherto considered to be an unique cause of the fall of the blood pH, showed no marked difference in its change, from those of the other experiments. Therefore it may be right to consider that the pH fall found at the 30th minute of this experiment was due to the influence of the low pH of the breathing water.

The rise of the CO_2-content which was observed after 30 minutes was also found in the other experiments, but from 1 hour afterwards it always decreased in the other experiments. While in the present case fishes A and B never decreased the highest CO_2-content, which was attained at the 30th minute of the experiment, until they died, and fish C, which showed no change by 1.5 hours later, raised this value in a great rate. Such connections have never been found in the other experiments and seem to be a marked distinction from the other experiments. This distinction may surely be due to the influence of the low pH of the breathing water.

In the other experiments hitherto conducted the change of the pH was paralleled with that of the CO_2-content in the gross. But in the present experiment the blood pH decreased rapidly with time, giving no increase on the way. While the CO_2-content showed no decrease but rather increased with time. Thus, regarding both the pH and the CO_2-content a marked distinction has been found between the acidosis caused by the O_2 deficiency and the acidosis which is caused by the low pH water.

The respiration frequency per minute was 86 to 114 at first. However it decreased with time, and after 30 minutes it became 55 and 40 in fishes A and B respectively. Fish C also showed a similar tendency, giving 45 after 1 and a half hours. The fact that the fishes once increased the respiration frequency within 20 or 30 minutes was a remarkable feature of the former experiments in which the acidosis was produced exclusively by the O_2 deficiency. In the present experiment, however, such increase was not recognized. This may be also a characteristic of the present experiment.

In contradiction to the experiments in which the fishes manifest notable weakness after 30 minutes, the fishes employed in this experiment showed no debility until 30 minutes later. But from 40 minutes

TABLE 47.

Case No.	Time	Air temp. (C)	Breathing water			Blood										Resp. freq.			Behaviour of fishes
			Temp. (C)	pH	O₂ (cc)	pH					CO₂ vol%								
						A	B	C	Mean	D	A	B	C	Mean		A	B	C	
1	0m	16.7	12.7	3.40	5.96	7.25	7.00	7.27	7.17	7.10	15.9	16.9	10.8	14.5		120	100	88	Excited.
2	5	17.0	12.8	3.40	5.861											90	100	86	Remained still on the bottom.
3	10	17.0	12.8	3.45	4.590											94	100	94	Breath regular
4	20	17.1	12.9	3.50	4.045											94	100	83	
5	30	17.1	13.0	3.53	3.542	6.82	6.77	6.75	6.78	7.12	22.5	20.6	21.5	21.5		90	88	79	Respire deeply.
6	40	17.2	13.1	3.60	3.167											100	86	73	Floated on the surface.
7	h m 1.00	17.4	13.2	3.60	2.834	6.77	6.76	6.55	6.72		23.5	22.5	23.3	25.1		91	79	60	C lay down; A and B weakened
8	1.30	18.0	13.5	3.65	2.834	6.75	6.76	6.65	6.72	7.12	24.4	19.6	22.2	25.4		89	77	48	B lay down.
9	2.00	18.4	13.7	3.67	3.231	6.65	6.70	6.63	6.66	7.11	27.4	17.6	21.5	22.2		79	79		C died. A and B breathed faintly.

afterwards they were gradually weakened and laid on the bottom after
1 hour. Thenceforth fishes A, B, and C died after 1 hour and 10
minutes, 1 hour and 40 minutes, and 2 hours respectively.

Leuciscus, Acidosis Experiment 8.
(May 21st, 9.20 am., 1927)

The present experiment was made with the object of verifying the
results of the preceding experiment The pH and the specific gravity
of the breathing water was made almost similar to those of the former
experiment. In the present experiment the breathing water was
aerated in order to prevent acidosis due to the O₂ deficiency.

After making a bleeding on each fish all were admitted into the
experimental jar at a time. Thereafter the bleeding was made at
intervals of 30 minutes, thus making 25 times of blood collection in all.

The measurements of 4 fishes used were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	35.3	7.2	493
B	33.7	7.4	471
C	35.8	7.6	518
D	35.4	7.9	510

(Table 47).

Leuciscus, Acidosis Experiment 9.
(May 22nd, 9 20 am., 1927).

An intention of altering the blood pH of *Leuciscus in vivo* was
successfully attained by the two preceding experiments. But in these
experiments the mode of the change of blood pH which occurs within
30 minutes was not observed. To make out this relation a further
experiment was attempted.

Although the general manner of the experiment was similar to
the former two experiments, the present one made in two times, using
1 fish and 10 litres of water each time. The tap water used for the
breathing of fishes had the pH 8.15 and the specific gravity 1.0237
(11°.9) which, however, decreased to pH 3.40 and specific gravity
1.0253 (11°.9) by an addition of n/10 HCl solution. For the purpose
of determining the blood pH accurately a particular saline solution

was prepared, making use of brom thymol blue as an indicator.

In each of the two successive tests bleeding was made at intervals of 5 minutes in the first 20 minutes, and thenceforth at intervals of 10 minutes until 40 minutes afterwards. Consequently 7 determinations were made on each fish. The measurements of the fishes were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	36.0	6.9	451
B	34.3	6.8	417

TABLE 48.

Fish	Case No	Time	Air temp (C)	Breathing water			Blood		Resp freq	Behaviour of fishes
				Temp (C)	pH	O₂ (cc)	pH	O₂ vol%		
Fish A	1	0m	13.1°	12.0°	3.40	6.667	7.15	16.6	94	Swam actively
	2	5	13.3°	12.0°	3.40	5.967	6.95	14.7	87	Still
	3	10	13.2°	12.0°	3.45	5.579	6.90	13.7	88	Breath regular
	4	15	13.4°	12.1°	3.45	5.198	6.85	14.7	83	,,
	5	20	13.4°	12.1°	3.50	4.998	6.85	14.8	86	Floated on the surface
	6	30	13.4°	12.1°	3.50	4.718	6.85	18.6	66	A little weakened
	7	40	13.4°	12.1°	3.50	4.761	6.85	19.7	83	Weakened.
Fish B	1	0	13.2°	11.9°	3.40	5.999	7.20	13.7	90	Excited.
	2	5	13.2°	11.9°	3.45	5.730	6.85	15.6	86	Quiet
	3	10	13.3°	12.0°	3.45	5.601	6.83	16.6	83	Respired deeply
	4	15	13.4°	12.0°	3.45	5.358	6.83	15.6	80	Remained still on the bottom
	5	20	13.4°	12.0°	3.50	5.439	6.83	16.5	77	,,
	6	30	13.4°	12.0°	3.50	4.923	6.80	19.6	86	A little weakened
	7	40	13.4°	12.1°	3.50	4.685	6.80	19.6	76	Breathed faintly.

We notice in the present experiment (as in the 2 preceding experiments), firstly, that the rise of pH which occured within the first 30 minutes of the Experiment No. 1 has not been observed; and, secondly, that the fall of the blood pH in the present experiment was recognized before the O_2 showed any marked change. These two facts distinctly characterise the present experiment, indicating that the acidosis can be produced by merely lowering the water pH without any and of

the O_2 deficiency. The following figure (Fig. 25) shows the change of the blood pH and CO_2-content in the present experiment.
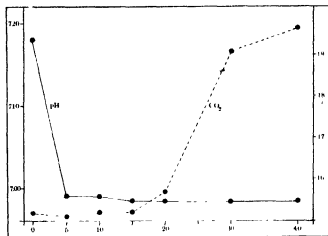

Fig. 25 Change of the pH and CO_2-content in blood (Leuciscus, Acidosis Exp 9)

Ordinate (left side) — pH
Ordinate (right side) — CO_2 vol%
Abscissa — time in minutes
Continued line — pH   Broken line — CO_2 vol%

Leuciscus, Acidosis Experiment 10
(May 24th, 9 20 am , 1927)

As can be seen from the result of the 2 preceding experiments Leuciscus lowers its blood pH and increase its CO_2-content when it was forced to breath in the acidified water (pH 3.40). Therefore I made another experiment with a view to know what further changes may occur in blood pH when the fishes were replaced in the tap water.

For the present purpose 4 fishes were kept in the low pH water after blood was collected from each fish. The second bleeding was made after 5, 10, and 20 minutes on fishes A, B, and C respectively. After the second bleeding the fishes were immediately replaced in the tap water. Thenceforth each fish was bled at intervals of 30 to 60 minutes until 3 hours afterwards, but after this it was bled at longer

TABLE 49.

Case No.	Time (h m)	Air temp. (C)	Breathing water Temp (C)	pH	O₂ (cc)	Blood pH A	B	C	D	Mean	Blood CO₂ vol % A	B	C	D	Mean	Resp. freq. A	B	C	D	Behaviour of fishes
1	0m	13.0°	11.6°	3.40	5.994	7.03	7.05	7.07		7.04	16.7	17.7	14.8	18.6	17.0	97	91	100	95	Excited
2	5	13.4°	11.7°	3.40	5.656			6.90		6.90			15.4		15.4	77	77	73	97	Still
3	10	13.4°	11.7°	3.40	5.558	6.58		6.86		6.86	16.7		16.8		16.8		77	73	97	Breath regular
4	20	13.6°	11.7°	3.40	5.601		6.70			6.70		14.8			14.8				66	"
5	30	13.6°	11.3°	8.02	5.547			7.23		7.23			14.6		14.6				66	All active
6	40	13.6°	11.3°	8.02	4.825															"
7	1.00	13.9°	11.3°	8.03	4.960	7.46	7.46	7.40		7.43	11.5	13.4	15.6		13.5	68	73	79	86	Still.
8	1.30	14.0°	11.3°	8.05	5.221		7.60	7.65		7.63		17.8	13.4	15.6	15.6		73	77	86	B weakened.
9	2.00	14.1°	11.3°	8.05	5.742		7.70	7.72	7.72	7.70			17.8		17.8	97		77	97	B nearly died
10	2.30	14.2°	11.3°	8.07	4.998			7.80		7.80			22.7		22.7		92		94	A, C, D vigorous.
11	3.00	14.2°	11.3°	8.07	4.976		7.81			7.81			17.7		17.7	78				"
12	3.30	14.1°	11.2°	8.07	5.342															"
13	4.00	14.0°	11.2°	8.08	4.939														90	"
14	5.00	13.9°	11.2°	8.10	5.052															"
15	6.00	13.8°	11.2°	8.10	5.159	7.87				7.87	20.7				20.7	56				"
16	24.00	12.9°	11.0°	8.12	4.512	7.65	7.45	7.37		7.46	16.7	21.6			21.6	83		69	81	"
17	48.00	14.2	11.3°	8.20	4.879	7.25	7.32	7.32		7.32	15.5	14	16.3		15.3	90	93	74	100	"

intervals, thus making 8 times of blood collection on each fish.

The pH of the breathing water was made 3.40, adding about 650 cc of n/10 HCl solution to 20 litres of sea water. The specific gravity which showed 1.0242 (11.2) at first fell to 1.0237 (11.2) after the addition of acid.

The measurements of the fishes employed were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	31.3	7.0	457
B	34 8	7.3	468
C	33.8	5.9	342
D	33.2	2.6	333

(Table 49)

B) ALKALOSIS

Leuciscus, Alkalosis Experiment 1.
(May 26th, 10 00 am . 1927)

The present experiment was conducted with the object of raising the blood pH of *Leuciscus* and observing the subsequent change of the CO_2-content when kept in the high pH water.

In the alkalosis experiment of *Cyprinus* the rise of the blood pH was so slow that it required 1.5 to 3 hours to exceed initial value. But in the present experiment it was anticipated that the pH rise might be very rapid in comparison with that of *Cyprinus*, because the effect of the low pH water upon the blood pH of *Leuciscus* was already found to be extremely rapid.

Though the procedure of the experiment was quite similar to that of the former experiments, the breathing sea water was made alkaline by the addition of NaOH solution. In the alkalosis experiments of *Cyprinus* the pH of breathing water became 9.80 when about 60 cc of n/10 NaOH solution was added to 20 litres of fresh water. While the breathing water used in this experiment needed about 260 cc of the above solution to raise the pH of the same volume of water to 9.80. This may, of course, be due to the buffer action of sea water. The decrease of the pH of breathing water during the experiment was not so rapid as during those of *Cyprinus*. So that it only needed some occasional addition of NaOH solution to prevent the depression

TABLE 50.

Case No.	Time	Air temp. (°C)	Breathing water Temp. (°C)	Breathing water pH	Breathing water O₂ (cc)	Blood pH A	Blood pH B	Blood pH C	Blood pH Mean	Blood CO_2 vol% A	Blood CO_2 vol% B	Blood CO_2 vol% C	Blood CO_2 vol% Mean	Resp. freq. A	Resp. freq. B	Resp. freq. C	Behaviour of fishes
1	0m	14.4°	11.9°	9.60	5.680	7.03	7.30	7.08	7.04	12.9	14.5	15.7	14.6	100	88	101	Somewhat weakened.
2	5	14.4°	12.0°	9.50	3.877										94	103	Remained still on the bottom.
3	10	14.4°	12.1°	9.40	2.965									104	95	105	
4	20	14.6°	12.1°	9.30	1.759												Respired deeply
5	30	14.6°	12.2°	9.10	0.766	7.55	7.53	7.30	7.43	11.5	11.5	16.2	12.7	115	90	103	Felt O₂ deficiency
6	40	14.6°	12.5°	9.05	0.754									80	60	75	All weakened
7	1.00 h	14.7°	12.5°	9.20	0.636	7.57	7.10	7.17	7.17	9.1	8.4	8.5	8.6	67	61	71	B floated upside down
8	1.20	14.8°	12.4°	9.15	0.571	7.03	7.00	7.01	7.01	9.1	6.0	8.3	7.8	56	46	63	A and C lay down.
9	2.00	15.0°	12.5°	9.10	0.742	7.00	6.80	6.85	6.88	8.0	6.0	8.3	7.4	0	0	0	All died.

of the pH of water. The initial pH of the sea water was 8.20 and
the specific gravity was 1.0270 (11°.9). However the specific gravity
fell to 1.0243 (11°.9) with the addition of NaOH solution.

Three fishes were used and the bleeding was made at intervals
of 30 minutes on each fish until it died. The dimensions of the fishes
were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	35.2	7.2	497
B	40.0	7.4	634
C	35.0	7.1	468

As will be seen in the above table a marked rise of blood pH
was observed in this experiment. Such a rise was also often observed
in the Acidosis Experiments (2-6). In addition to this the pH change
observed 1 hour afterwards also closely resembled those of the acidosis
experiments. Therefore the pH change found in this experiment may
be considered as depending upon the O₂ decrease but not upon the
high pH of the breathing water.

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Leuciscus, Alkalosis Experiment 2.

(May 28th, 9 00 am, 1927)

</div>

On account of the decrease of O₂ content in breathing water the
aim to change the blood pH and CO₂-content has not been attained
in the former experiment. So that I conducted here one more experi-
ment keeping both the pH and the O₂-content still higher.

In the present experiment fresh water was used for the breathing
of fishes. The pH of the water rose to 11.00 by adding 40 cc of
n NaOH solution to 20 litres of tap water. To prevent the decrease
of O₂ the breathing water was renewed every 10 minutes until 40
minutes later and thereafter it was not renewed until the experiment
ended. The bleeding was made every 30 minutes. Because of the
marked debility of the fishes blood collection was made but 3 times
on each fish.

The demensions of the fishes were as follows.

TABLE 51.

Case No.	Time	Air temp. (°C)	Breathing water Temp. (°C)	Breathing water O₂ (cc.)	Breathing water pH	Blood pH A	B	C	Mean	Blood CO₂ vol% A	B	C	Mean	Resp. freq. A	B	C	Behaviour of fishes
1	0m	15.6°	11.7°	6.706	11.0	7.02	7.01		7.03	15.0	19.9	16.8	17.2	87	83	111	Excited.
2	5	15.1°	11.7°	5.538	—									86	75	87	Gentle.
3	10	15.2°	11.7°	6.166	11.0									96	79	97	"
4	20	15.3°	11.8°	4.847	11.0									100	75	70	Agonized.
5	30	15.3°	11.8°	5.071	11.0	7.35	7.35	7.31	7.32	11.7	11 7	14.9	12.8	94	77	86	Somewhat weakened.
6	40	15.3°	11.6°	5.681	—									43	40	75	All floundered.
7	1.00 h m	15.3°	11.7°	5.825	—	7.25	7.05		7.11	10.9	11.5	11.5	11.3	41	57	64	All nearly died.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	36.9	7.6	573
B	38.1	7.6	623
C	34.0	6.8	432

The O_2 decrease in this experiment was too little to produce acidosis. Therefore the rise of the pH observed above may possibly be attributed to the effect of high pH water.

As the consequence the fish died much sooner than in any of the former experiments, in spite of the frequent renewal of the breathing water. This shows that the death of the fishes were not caused by the O_2 deficiency but it must have resulted from the high pH of the breathing water. In the present experiment some outstanding features which were not found in the other cases was observed. For instance, fishes excreted a considerable amount of mucus from the body and the gill surface. From 30 minutes afterwards the amount of mucus secreted immensely increased and at last it seemed as if the fishes were enclosed in a mucilaginous media

This experiment suggests that the high pH exceeding 11.00 is too serious to the fishes for conducting the experiment.

Leuciscus, Alkalosis Experiment 3.
(May 30th, 9.30 am , 1927)

The preceding two experiments failed in the aim of raising the blood pH of Leuciscus, because of the O_2 dificiency of the water in one case and of an excessively high pH of water in another case. Therefore the present experiment was carried out so as not to decrease the O_2 content on the one hand, and to keep the water pH moderately on the other hand.

About 26 cc of n NaOH solution was added to 20 litres of breathing sea water, thus raising its pH to ca 9.70.

The renewal of water was made every 30 mintes at first, and less frequently thereafter, thus changing the breathing water 19 times in all. After the rise of blood pH was accurately determined at the end of the 2nd hour, the fishes were replaced in the tap water with a hope of recovering the normal blood pH. The tap water used in

TABLE 52.

Case No.	Time	Air temp (C)	Breathing water Temp (C)	Breathing water pH	Breathing water O₂ (cc)	Blood pH A	Blood pH B	Blood pH C	Blood pH Mean	Blood CO₂ vol's A	Blood CO₂ vol's B	Blood CO₂ vol's C	Blood CO₂ vol's Mean	Resp freq A	Resp freq B	Resp freq C	Behaviour of fishes
1	0 m	16.7	13.7	9.70	5.698	7.05	7.65		7.33	18.1	16.2	15.0	16.4	126	106	113	Vigourous.
2	5	16.7	13.7	9.60	6.464									130	98	75	Still.
3	10	16.8	13.6	9.60	5.573									136	86	85	"
4	15	17.2	13.4	9.60	4.751									136	86	100	Active
5	20	17.2	13.4	9.60	4.773									130	83	94	"
6	25	17.2	13.4	9.60	4.777									138	85	90	"
7	30	17.2	13.4	9.60	4.762									160	100	78	"
8	35	17.2	13.4	9.60	4.784									160	103	100	"
9	40	17.2	13.4	9.60	5.299									133	100	167	"
10	45	17.2	13.4	9.60	5.156									120	103	83	"
11	50	17.2	13.4	9.60	4.996									104	100	103	"
12	55	17.3	13.4	9.60	5.272									120	104	111	"
13	1.00	17.5	13.4	9.00	5.316	7.62	8.05		7.92	14.3	25.1	23.4	20.9	130	104	91	"
14	1.05	17.5	13.4	9.00	—												A little agonized.
15	1.10	17.4	13.4	9.00	—												"
16	1.15	17.5	13.4	9.00	—									111	100	100	"
17	1.20	17.6	13.4	9.00	5.247									133	120	100	"
18	1.25	17.7	13.4	9.00	5.116									120	120	104	"
19	1.30	17.8	13.4	9.00	5.702									120	120	104	Still
20	1.35	17.8	13.4	9.00	5.640									120	120	104	"
21	1.40	18.0	13.4	9.70	5.294									108	94	88	"
22	1.45	18.0	13.4	9.70	5.177									91	91	93	"
23	1.50	18.0	13.4	9.70	6.197	7.80	8.10		7.86		24.2	19.3	20.5	125	94	100	"
24	1.55	18.1	13.4	9.70	5.228									117	92	94	"
25	2.00	18.1	13.4	9.70	5.474	7.70	8.97		7.99	18.1	24.3	22.1	23.4	107	99	100	"
26	3.00	18.5	13.6	8.10	6.146									142	73	73	"
27	4.00	18.6	13.7	8.10	4.660	7.65	7.85	7.85	7.80	24.1	22.1	18.1	20.0	47	95	88	Active
28	6.00	18.1	13.7	8.15	5.591	7.45	7.83		7.71		21.2	18.1	17.2	86	95	81	"
29	8.00	18.1	13.5	8.12	5.600		7.87		7.72		21.5	18.1	17.2	90	83	77	"
30	24.00	16.7	13.4	8.10	5.134	7.43	7.50	7.48	7.48		19.9	15.0	16.6	90	100	110	"

this experiment showed its pH and specific gravity to be 8.20 and 1.0235 (13°.6) respectively.

The bleeding was made 7 times on each fish within 48 hours. The former experiments showed us that the fishes mostly raise their blood pH after 30 minutes unless the pH of water is decreased unusually. Therefore no determination was made till 1 hour later. After this the bleeding was made at the end of 1, 2, 4, 5, 24 and 48 hours successively.

The dimensions of the fishes employed were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	36.1	6.9	482
B	34.5	7.3	502
C	35.0	7 4	513

The above results lead us to conclude that *Leuciscus* should have exhibited an apparent alkalosis when it breathes in the high pH water (above 9.60), had it not felt the O$_2$ deficiency.

Leuciscus, Alkalosis Experiment 4.
(June 1st, 9 30 am , 1927)

From the results of the former experiment it was found that the blood pH of *Leuciscus* remarkably rose by 1 hour later when the fish respired in the high pH water. Therefore a further determination was made to find the rate of the pH rise within 1 hour.

As in the preceding experiment the pH of the breathing water was made 9.70 and the water was renewed every 5 minutes till 2 hours later, so that the O$_2$ content of the water was kept moderately high. The pH and the specific gravity of the tap water were 8.20 and 1.0234 (13".3) respectively. 3 fishes were used and the bleeding was made every 20 minutes till 1 hour later. Thenceforth, the bleeding was made at intervals of 30 minutes, thus making 6 times of blood collection on each fish by 2 hours afterwards.

The measurements of the fishes used were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	36.2	7.0	512
B	35.6	6.5	360
C	33.8	6.3	412

TABLE 53.

Case No.	Time	Air temp (°C)	Breathing water Temp (°C)	Breathing water O₂ (cc)	Breathing water pH	Blood pH A	Blood pH B	Blood pH Mean	Blood pH C	Blood CO_2 vol% A	Blood CO_2 vol% B	Blood CO_2 vol% C	Blood CO_2 vol% Mean	Resp freq A	Resp freq B	Resp freq C	Behaviour of fishes
1	0 cm	15.6°	13.8°	6.697	9.70	7.00	7.01	7.00	7.00	13.0	13.0	20.1	16.4	91	105	100	Excited.
2	5	15.4°	13.4°	6.316	9.60									82	88	80	Still.
3	10	15.8°	13.4°	6.294	9.60									83	83	91	Active.
4	20	15.8°	13.8°	4.911	9.60	7.30	7.03	7.21	7.30	17.0	14.1	13.3	14.8	90	90	74	„
5	30	16.0°	13.8°	5.071	9.60									89	100	75	Normal.
6	40	16.0°	13.8°	5.106	9.60	7.42	7.30	7.41	7.52	14.2	14.1	16.5	14.9	90	120	76	„
7	1.00 h m	16.0°	13.8°	5.941	9.60	7.43	7.34	7.46	7.60	17.0	12.4	18.9	16.1	90	104	82	Fish A somewhat weakened.
8	1.30	16.2°	13.8°	5.928	9.60	7.43	7.34	7.46	7.60	12.0	13.8	18.9	14.7	91	103	88	Fish A upside down.
9	2.00	16.3°	13.8°	6.708	9.60	7.60	7.92	7.93	7.46	17.3	14.1	21.2	17.5	—	100	88	Fish A breathed intermittently.

The results of the present experiment are, in brief, that the rise
of blood pH due to the high pH of breathing water begins so soon
as 20 minutes from the beginning of the experiment.

Leuciscus, Alkalosis Experiment 5.
(June 3rd, 9 29 am, 1927)

In the former experiment the change of CO_2-content which took
place in association with the change of blood pH has not been clearly
determined. The present experiment was made with a hope of making
clear this relation.

The manner of experiment was almost the same as in the former
case (Exp. 4). As the breathing water was renewed every 5 minutes
the effect of the O_2 deficiency was not exhibited until 3 hours later
After this the fishes transferred into the tap water with the hope
of recovering their vigour. The bleeding was carried out 8 times on
each fish at varying intervals. The initial pH and the specific gravity
of the water were 8 20 and 1 0235 (13 .3) respectively

The measurements of the 3 fishes employed were as follows

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	34.4	6.0	368
B	35.2	6.4	418
C	36.0	6 6	477

(Table 54)

Leuciscus, Alkalosis Experiment 6.
(June 4th, 10.00 am, 1927)

The results of 16 experiments hitherto conducted on *Leuciscus* led
us to conclude that the pH of this fish rises in a comparatively short
time, either with high or low pH of the breathing water. Therefore
I made a further experiment in which it was tried to cause an acidosis
and alkalosis alternately.

The method of experiment was similar to that of the former
experiment, renewing the breathing sea water every 5 minutes. For
the acidosis the pH of water was made 3.70 and for the alkalosis it
was made pH 9.70. The fishes were placed in the high pH water
until 1.5 hours later. After raising the blood pH in this manner they

TABLE 51.

Case No.	Time	Breathing water				Blood								Resp freq			Behaviour of fishes
		Air temp. (C)	Temp (C)	pH	O₂ (c.c.)	pH				CO₂ vol.%							
						Mean	A	B	C	Mean	A	B	C	A	B	C	
1	0m	14.6°	13.3°	9.70	5.272	7.25	7.20	7.30	7.25	17.5	16.8	18.8	17.8	111	111	111	Excited.
2	5	14.8°	13.3°	9.60	4.244									90	100	94	Still.
3	10	14.5°	13.3°	9.60	4.117									94	110	106	Breath regular.
4	20	14.3°	13.4°	9.00	2.991									97	105	106	Very still.
5	30	14.4°	13.5°	9.60	4.485	7.52	7.48	7.55	7.43	16.8	14.8	19.6	17.8	109	107	107	״
6	40	14.4°	13.4°	9.60	4.525									109	106	106	״
7	1.00 h.m	14.8°	13.4°	9.60	4.271	7.76	7.75	7.83	7.70	16.0	14.5	15.3	17.8	85	97	97	״
8	1.30	15.0°	13.4°	9.60	4.466	7.82	7.90	7.90	7.80	20.0	20.5	19.8	19.3	94	104	107	״
9	2.00	15.0°	13.4°	9.60	4.434	7.86	7.86	7.90	7.87	16.1	14.3	21.9	12.3	85	97	103	״
10	2.20	15.0°	13.4°	9.60	4.792									86	115	111	A little agonized
11	3.00	15.2°	13.4°	9.60	5.284	7.70	7.73	7.46	7.72	20.1	20.3	18.8	21.7	104	111	104	Somewhat weakened
12	8.00	15.0°	13.6°	8.15	5.616	7.70	7.55	7.45	7.80	25.3	20.9	22.7	22.3	72	97	90	Recovered
13	24.00	15.0°	13.6°	9.13	5.297	7.62	7.85	7.55	7.47	18.1	19.7	21.8	12.8	72	113	87	Normal

TABLE 55.

Case No.	Time	Air temp. (C)	Breathing water			Blood									Resp. freq.			Behaviour of fishes
			Temp. (C)	pH	O₂ (cc)	pH				CO₂ vol%					A	B	C	
						A	B	C	Mean	A	B	C	Mean				
1	0m	14.3	13.6	9.70	5.680	7.03		7.32	7.13	12.8	18.8	14.3	15.3		107	115	111	Active
2	.20	14.6	13.6	9.60	5.490										120	111	107	Suil
3	h m 1.00	14.8	13.7	9.60	5.468	7.42	7.85	7.48	7.58	13.4	18.3	16.8	16.2		107	100	97	"
4	1.30	15.3	13.7	9.60	5.722	7.53	8.15	7.73	7.78	16.6	24.7	18.4	19.9		107	97	85	"
5	1.40	15.8	13.7	3.70	5.669	7.23	7.05	6.95	7.08	21.5	24.0	20.0	21.8		107	81	88	Fishes floundered a little
6	2.10	16.0	13.7	3.70	5.384	6.75	6.76	6.75	6.75	21.8	23.0	22.4	22.4		46	75	60	C died soon after.
7	2.40	16.0	14.2	9.60	6.072	7.43	7.60		7.47	23.0	24.1		23.6		104	100		Other fishes recovered.
8	3.40	16.0	14.2	9.60	6.292	7.47	7.55		7.51	23.0	24.9		24.0		103	100		Normal.

were transferred into the low pH water and forced them to respire the acid water for 30 minutes, i. e., until 2 hours and 10 minutes later. As they lowered the blood pH pronouncedly until this time they were again introduced into the high pH water. Then after determining the secondary rise assuredly, the experiments were discontinued.

Bleeding was once made before they were admitted in the high pH water and after the admission they were bled twice, after 1 and 1.5 hours. After they were transferred into the low pH water the bleeding was made twice, after 10 and 30 minutes. Thenceforth further bleeding was made twice, 30 minutes and 1 hour later, after the replacement of the fishes into the high pH water. Consequently 7 times of blood collection were made on each fish, except on one which died during the experiment.

The measurements of the fishes were as follows.

Fish	Body length (cm)	Body height (cm)	Body weight (gms)
A	42.0	7.0	474
B	48.0	6.8	492
C	34.5	6.7	402

(Table 55).

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Changes in the Blood Picture, and in the Oxygen Capacity of the Blood Haemoglobin of the Carrier-Pigeon following Splenectomy.

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(With 7 Text-figures)

INTRODUCTION

The blood changes after splenectomy have been exhaustively studied by many workers, but one phase of the ploblem, that bearing on the possible influence of the splenectomy on the character of the blood pigment, has not been thoroughly explored. STIMSON ('27) discovered that the splenectomy in rabbits causes the appearance of a non-oxygen-carrying haemoglobin in the circulating blood. As far as I know, however, there is no one who has studied the changes in the blood haemoglobin of birds following splenectomy.

In the present investigation I have dealt with the relation between the oxygen capacity of the blood haemoglobin and the blood picture of the carrier-pigeon following splenectomy, with the hope of obtaining further data concerning the physiological function of the spleen.

MATERIALS AND METHODS.

The material used in my experiments was the carrier-pigeon. All specimens employed were healthy adults varying in weight from 250 to 300 gms.

The surgical technique of the splenectomy was simple. Clean and sterile instruments were used. The animals were lightly etherized, and the skin and the lateral body wall of the right side were opened. The stomach was seized with · forceps and pulled out through the small incition untill the spleen appeared. The spleen was extirpated and the stomach replaced. Preceding the extirpation of the spleen,

it was strictly necessary to ligate the splenic artery at the entrance of the vessel to the spleen, in order to avoid unnecessary loss of blood. The body wall and the skin were sewn. Only one of the specimens became septic after operation.

The oxygen capacity of the blood was determined by VAN SLYKE's method. About 0.5 cc. of the blood, at a time, per specimen, was taken from the branchial vein by inserting the injection needle into the vessel. An initial sample was taken 1 or 2 days before splenectomy and further samples were obtained at varying intervals afterward. The samples were defibrinated and kept in a porcelain dish and were used within 3 hours after removal from the body, since the afinity for oxygen of haemoglobin gradually diminishes. In my experiment, 0.2 cc. of the sample was introduced into a pipette of fifteen cc capacity and equilibrated with atmospheric air by means of KATO's micromethod in a water bath at a temperature of 11°C, and then was drawn into the apparatus for analysis.

The methods employed in the blood picture were as follows:

The blood was taken from the branchial vein and a THOMA-ZEISS Haemometer was used for the enumeration of the corpuscles. For the haemoglobin estimation, SAHLI's Haemometer was used. Differential leucocyte count was made from the ordinary blood spread, as is usual in such experiments.

RESULT OF THE EXPERIMENT.

I have preliminarily tested my own data on the enumeration of the erythrocytes in the chick blood and compared the results with those found by several other investigators. The results of enumeration are shown in the following table.

Materials	No of erythrocytes	Investigators.
Chick	2.100.000	KOHANAWA
,,	2.400.000	HYEN
,,	3.080.000	TORYU
,,	3.100.000	MALASSEZ
,,	3.860.000	STÖLZING
Carrier-Pigeon	4.650.000 (20 specimens)	TORYU

As will be seen from the above table, my own result obtained in

TABLE I.

Erythrocyte and leucocyte count after splenectomy.

Days after splenectomy	No of total red cells	No. of normal red cells	No of poly-chromatophiles	No of leucocytes
		Before splenectomy		
	4.549.000	4.549.000	0	31.875
		After splenectomy.		
1	4.000.000	3.772.000	228 000	40.600
2	3.940.000	3 778.000	162.000	43.500
5	3 515.000	3.196.000	329.000	43.800
7	3.464.000	3.298.000	267.000	52.500
10	3.595.000	3.279.000	316.000	52.500
13	4.212.000	3 770.000	442.000	40.600
16	4.280.000	4.211.000	69.000	37.500
21	4 560.000	4.513.000	47.000	25.000
24	4.850.000	4.800 000	50.000	21.900
29	4.600.000	4.572.000	28.000	37.500
34	5.000.000	4.961.000	54.000	28.100
50	4.628.000	4.599.000	29.000	28.200

TABLE II.

Haemoglobin content in the blood of the carrier-pigeon after splenectomy.

Days after splenectomy	Haemoglobin content in total red cells	Haemoglobin value	Expected value of haemoglobin content in normal red cells
		Before splenectomy	
	19.03	1.00	19.03
		After splenectomy.	
1	17.17	1.02	16.19
2	13.63	0.86	13.10
5	13.67	0.93	12.45
7	12.28	0.88	11.60
10	13.49	0.86	12.85
13	14.19	0.80	12.70
16	14.19	0.79	13.93
21	16.44	0.86	16.27
24	18 16	0.91	17.07
29	17.30	0.89	17.20
34	18.16	0.87	18.02
50	18.68	0.95	18.57

the chick agrees with those given by other workers, and therefore
the higher value found in the carrier-pigeon is due not to technical
error but to the specificity of the pigeon used.

TABLE III.

Differential leucocyte count after splenectomy.

Days	Total leuco-cyte count	Differential leucocyte count (%)				
		Lympho-cytes	Large mono-nuclears	Eosin-ophiles	Pseudo-eosinoph.	Basophiles
		Before splenectomy.				
	31.900	55.33	10.00	2 67	30.00	2.00
		After splenectomy				
1	40.600	13.44	3.27	1.63	80.98	0.66
2	43.800	26 33	14 00	5.00	54.00	0.67
5	43.800	59.00	4 67	5.00	30.67	0.67
7	52.500	27.33	3 90	0.90	67.89	0.90
10	52.500	14.75	4.25	0.75	79.75	0.50
13	40.600	34.33	5 00	0.33	59.33	1.00
16	37.500	36.00	4 34	1.33	58.00	0.33
21	25.000	42.00	4.33	5.67	46.33	1.67
24	21.900	38.00	6 49	4.59	48.92	1.89
29	37.500	23.89	3.89	4.17	66.94	1.11
34	28.100	11.56	4.06	4.06	78.13	2.19
50	28.200	10.52	4.51	4.54	78.12	2.81
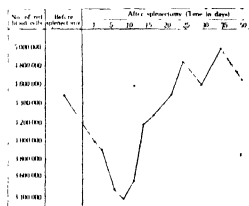
Fig. 1   Composite curve of the red blood cell counts of
5 carrier-pigeons after splenectomy.
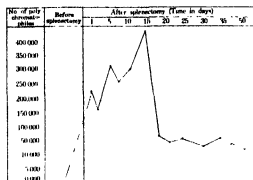
Fig. 2. Polychromatophile counts after splenectomy. (Average of 5 carrier-pigeons).
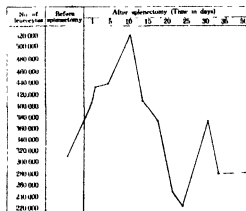
Fig 3 Leucocyte counts after splenectomy. (Average of 5 carrier-pigeons).
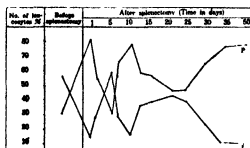
Fig. 4. Differential leucocyte count after splenectomy. (Average of 5 specimens).
    L. Lymphocyte counts.
    P. Pseudoeosinophile counts
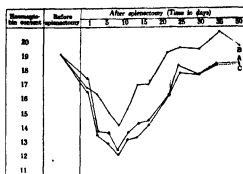
Fig 5 Showing the relation of the haemoglobin content
to the red cell counts after splenectomy

△——△ A   Haemoglobin content at every instance.

●——● B   Haemoglobin content against the number of
total red cell.

○——○ C   Haemoglobin content against the number of
normal red cells

1.   Result Obtained for the Blood Picture following Splenectomy.

The full test of each experiment is not given, but I may add that
the range of variation was very small (±1.944%) throughout the entire
series of experiments. I have given the numerical data of one case
only in Table I, and that of others are shown only in curves.

The splenectomy in the carrier-pigeon, as is shown in Table I and
Fig. 1, produces an immediate decrease in the number of erythrocytes
(often 25 per cent or more). But in about a week the erythrocytes
begin to increase and in about 3 weeks usually reach the number
before the splenectomy, and in about 7 weeks become more numerous
than before the operation. This confirms the results of MOLE ('25)
on the rabbit.

As is also shown in Table II and Fig. 5, the haemoglobin content
decreases as the erythrocyte count becomes lowered, but the degree
of the reduction is much greater than the reduction of red cells, even
when the red cell number has become more numerous than before
the operation, the haemoglobin content does not reach a normal state,
showing 0.95 of haemoglobin value at the highest, except in 1.02 at
the first day after the operation. Consequently, the haemoglobin
content in every instance (curve A in Fig. 5) is always less than that

against the number of the total erythrocytes (curve B in Fig. 5).

Splenectomy in the carrier-pigeon, as will be seen from Table I and Fig. 2, causes an appearance of the polychromatophiles in from about 0.5 to 10.0 percent of the total red cells. The number of these cells shows a gradual increase with the progress of anemia up to 13 days after splenectomy, probably due to the rapid formation of erythrocytes. In about two weeks, when the erythrocytes begin to increase, the polychromatophiles suddenly diminish, but nerver disappear throughout the entire period of anemia of the animal, following splenectomy.

Histologically, the polychromatophiles are somewhat larger than the normal erythrocytes and take the basophile tint. So I suppose, as has been suggested by WALKER ('04) the polychromatophiles are younger forms than the normal erythrocytes and contain a lesser amount of haemoglobin. To support the view just stated I notice the following relation: first, the degree of the reduction of the haemoglobin content is proportionately greater than the reduction of red cell count as already mentioned; second, as will be seen from Fig. 5, the haemoglobin content in total red cells at every instance after the operation almost agrees with the expected value of haemoglobin content when the normal red cells alone are considered as functional. If the last relation is granted, it becomes highly probable that the polychromatophiles contain the least amount of haemoglobin. We will concider this question again under the section "Oxygen capacity after splenectomy."

A decrease in the number of the erythrocytes and in the haemoglobin content, which is characteristic of anemia following the extirpation of the spleen, is thus true in the carrier-pigeon. The duration of anemia following splenectomy varies in different animals. PEARCE shows, on the dog, that anemia lasted about 150 days, while the recent work of MOLE ('25) on the rabbit, shows that the duration of anemia was from 4 to 8 weeks. FUKUDA ('24) reports on poultry that anemia lasted 24 days, OHUYE ('27), on the newt, 10 to 60 days.

In my experiment on the carrier-pigeon, the recovery of the number of the red cells to normal is reached within from 10 to 20 days, but the haemoglobin value does not reach the normal level even when the red cell number has become more numerous than before the

operation, due probably to the appearance of polchromatophiles, as mentioned above. This suggests that the spleen may play a part in the preparation of new haemoglobin, or in the preservation of the iron set free by the death of the red corpuscles and supplied from diet. GROSSENBACHER and ASHER ('08) found that after extirpation of the spleen there was a distinct increase in the daily loss of iron from the body, in dogs, an increase from 11 to 18 mgm. Whether or not a similar result occurs in the carrier-pigeon needs further investigation to determine

Table III and Fig. 3 show an invariable increase in the number of white cells within a few days of splenectomy; after which there is gradual decrease, the normal level being reached in 30 days.

I suppose the increase in the number of white cells which occurs immediately after the operation is a post-operative leucocytosis.

As is shown in Table III, the lymphocyte count decreases with the progress of time after the operation, while other elements, especially the pseudoeosinophile shows strictly the reverse relation. Consequently, the curves L and P in Fig. 4. are obtained.

A decrease in the number of lymphocytes in the blood of mammals following splenectomy has not been recognized, because of the fact that the lymphatic glands or nodes supply the loss by the production of lymphocytes. On the contrary, however, since the lymphatic glands are lacking in the carrier-pigeon, as in some other birds, the extirpation of the spleen would bring about a decrease in the number of lymphocytes.

II. Result Obtained for the Relation Between the Haemoglobin Content and the Oxygen Capacity Following Splenectomy.

The results of the investigations for the haemoglobin content and the oxygen capacity before and after the splenectomy are given in Table IV and are shown graphically in Figs. 6 and 7.

The oxygen capacity of the haemoglobin in every instance after the operation showed more or less reduction when compared with the oxygen capacity shown by the total haemoglobin given by the control pigeon. This indicates that the splenectomy in the carrier-pigeon causes either the appearance of inactive red cells which are incapable of taking on oxygen in the circulating blood, or reduced ability of combining with oxygen in all the red cells. We have, however,

TABLE IV.

Haemoglobin content and oxygen capacity following splenectomy.

Days after splenectomy	I. Total haemoglobin content at every instance	II. Expected O₂-carrying haemoglobin from observed O₂ capacity (IV)	III. Non-O₂-carrying haemoglobin in total haemoglobin content (I-II)	IV. Observed O₂ capacity for given total haemoglobin content	V. Theoretical O₂ capacity of haemoglobin which is made equilibrated to the control.
			Before splenectomy.		
	19.03	19.03	0.00	31.20	31.20
			After splenectomy		
1	17.17	16.74	0.43	27.45	28.15
2	13.67	13.30	0.37	21.80	22.41
5	13.67	11.60	2.07	19.63	22.41
7	12.28	9.80	2.48	16.07	20.13
10	13.49	11.05	2.44	18.11	20.12
13	14.19	14.00	0.19	22.95	23.21
16	14.19	14.18	0.01	23.25	23.25
21	16.44				26.85
24	18.16	16.81	1.35	27.56	31.34
29	17.30	17.91		29.36	28.36
34	18.16	17.29	0.87	28.34	31.34
50	18.68	18.17	0.51	29.68	30.06
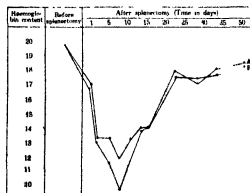
Fig. 6. Showing the relation between the haemoglobin content and the oxygen-carrying haemoglobin after splenectomy.

  A. Total haemoglobin content.

  B. Oxygen-carrying haemoglobin.
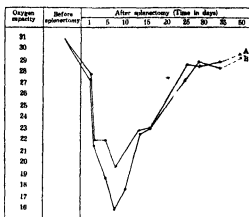
FIG. 7   Showing the relation between the oxygen capacity
against oxygen-carrying haemoglobin and that against the total
haemoglobin.
A.   Oxygen capacity against total haemoglobin.
B   Oxygen capacity against oxygen-carrying haemoglobin.

assumed already that this reduction is more probably due to the
appearance of polychromatophiles than to the general falling of the
oxygen-combining ability of all red cells, from the fact that the cells
under consideration are younger and that further more, the haemo-
globin content is relatively less when these cells increase, and vice
versa.

The amount of this inactive haemoglobin, as will be seen from
Table IV and Fig. 6, gradually increases with the progress of anemia,
reaching the maximum about 2 weeks after the operation, when it
begins to decrease, and in about 7 weeks almost disappears.

STIMSON ('27) found in splenectomized rabbits a non-oxygen-carrying
haemoglobin, from 4 to 20 percent of the total haemoglobin, which
disappeared in 2 or 3 days after the operation.   By my own obser-
vation in the carrier-pigeon, the inactive haemoglobin is from about
I to 25 percent in amount and is of much the same order as that
found in the rabbit by STIMSON just stated, but the duration of its
appearance in the carrier-pigeon is far longer, appearing throughout
almost the entire period of anemia.

STIMSON considered the inactive haemoglobin found in his splenec-

tomized rabbit to be the haemoglobin derivative which has methaemo-
globin like properties and is capable of being activated, in terms of
oxygen capacity, by a strong reducing agent. Further work is being
undertaken to obtain more information, if possible, as to the nature
of the non-oxygen-carrying haemoglobin found in the blood of the
carrier-pigeon following splenectomy.

SUMMARY.

The results obtained in this investigation may be summarized as
follows :

1. Removal of the whole spleen in the adult carrier-pigeon usually
at once produces a decrease in the number of erythrocytes. In about
a week they begin to increase and in about 7 weeks are usually more
numerous than before the operation.

2. The erythrocyte decrease is associated with a considerable
polychromatophile increase during the first 2 weeks, suggesting increased
haemopoiesis.

3. The haemoglobin contents at every instance after splenectomy
show lower values than those against the total erythrocyte counts.

4. Removal of the whole spleen in the adult carrier-pigeon pro-
duces an increase in the number of leucocytes, suggesting a post-
operative leucocytosis. They return to normal after about 2 weeks.

5. There are noteworthy changes concerning the differential leuco-
cyte count : The number of lymphocytes decreases with the progress
of time after the splenectomy, while that the pseudoeosinophile shows
strictly the reverse relation.

6. Splenectomy in the adult carrier-pigeon causes the appearance
of a non-oxygen-carrying haemoglobin varying in amount from 1 to
25 percent of the total blood haemoglobin. This inactive haemoglobin
seems to be associated with the appearance of polychromatophile cells.

Before leaving the subject, I wish to express my hearty thanks to
Prof. S. HATAI for his valuable suggestions and criticism throughout
the entire course of this work.

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On the Circulation of the Periviseral Fluid in *Caudina chilensis* (J. Müller).[1]

By

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(With 2 Text-figures)

The periviseral cavity of *Caudina* is filled with pink colored fluid and by it all the internal organs, such as alimentary tract, respiratory trees, gonads, and so on, are bathed. This color of the fluid is due to the presence of haemoglobin containing red blood corpuscles. The periviseral fluid appears to casual observers stagnant and without current except a general movement as the animal contracts or retracts its body.

As early as 1896, GEROULD made an observation on the circulation of *Caudina* within the tentacles, but of the periviseral circulation very little was stated

WIDMARK (1911) who made many experiments on *Aurelia* and *Cyanea*, found that the dissolved substances in periviseral fluid were distributed well by the currents caused by the movement of cilia lining the whole peritoneum. So far as I am aware very little is known concerning the general circulation of the periviseral fluid about Echinoderms or with the pressure of the body fluid under varied conditions. Hence I undertook, at the suggestion of Professor Dr. S. HATAI, the problem of the circulatory current of the body fluid of *Caudina chilensis* (J. Müller), materials for which can be found in large numbers in the sandy shores near the Asamushi Marine Biological Station.

1. THE MOVEMENT OF TENTACLES

A sand burrowing by *Caudina* is solely accomplished by the well co-ordinated movement of the tentacles and indeed the survival of *Caudina* almost depends on the proper adjustment of the tentacles.

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[1] Contributions from the Marine Biological Station, Asamushi, Aomori-ken No. 54

YAMANOUCHI (1929) who made very careful observations on the behaviour of *Caudina* states that " the tentacles are the most important organ for movement in the sand, and swallowing the sand by the aid of tentacles would much facilitate its movement in addition to its food taking "

The tentacular activities of *Caudina* are well described by GEROULD (1896) and I shall quote some of his words : " *Caudina* burrows head foremost mainly by means of its tentacles, which by the alternate contraction of their outer and inner longituginal muscles move back and forth in a radinal direction, crowding aside the grains of sand which lie in its course. A forward movement is facili ated by the animal swallowing the sand immediately in front of it, as is said to be the case also with *Synapta* and many worms ".

The projection or expansion of the tentacles is, no doubt, produced by the pressure of the intra-tentacular fluid acting on the elastic membranous wall, but how strong is its pressure is as yet not known, and I have thus first attempted to determine its value. However it is difficult to determine the pressure in tentacular fluid directly from the tentacles because the tentacular ampulla is the essential regulator of the pressure of the fluid since it is continuous to the water canal system. I have therefore measured the pressure of the ampulla instead, by inserting a delicate injecting needle, to which a manometer of apropriate size is attached, and obtained the following range of figures from 10 specimens.

<center>13–25 cm/water</center>

The above figures were obtained from the *Caudina* immediately after the body was opened and it is therefore probable that the pressure of the fluid within the tentacles will be much higher in normal intact *Caudina* than the values shown in the above.

2. THE PRESSURE OF PERIVISCERAL FLUID.

The perivisceral wall of Holothurians is remarkably efficient as compared with that of other Echinoderms, since in the former the perivisceral wall not only possesses properties of spontaneous rythmic movement but also contractive or retractive power of the entire wall.

The increase of contractile force of the body wall produces increase

in pressure of the perivisceral fluid, and during violent contraction of
the animal the pressure becomes accordingly very great.

The following figures which were obtained from ten *Caudinas* in
each experiment show the pressures of the perivisceral fluid in *Caudina*
(adult form) at 18°C sea water under varied conditions:

    10–20 cm/water; normal condition or at rest in sand,
    30–40 cm/water; sand burrowing animals,
    45–50 cm/water; contraction by electrical stimulus (2 Volts) applied
                    on the body surface for less than a second, and
    45–50 cm/water; contraction by elect. stim. (4 Volts).

The figures given above show that the perivisceral pressure under
the working condition is more than double that of the animal in a
resting condition. The pressure during the contraction of the body
caused by an electrical stimulus shows 2.5–4.5 times the pressure shown
during the resting condition. It will be noted however that a much
stronger electrical stimulus and consequently much greater contraction
of the body is not accompanied by a proportionately greater pressure,
indicating that 45–50 cm/water is probably the maximum pressure
attainable by the adult *Caudina*.

The pressure of the perivisceral fluid in the younger *Caudina*
(about 10 mm. in length) is considerably lower than that given by the
adult form. In the average, the pressure at rest is only about 2–4 cm.
of water and even during the stronger contraction of the body, pro-
duced either by mechanical or by electrical stimuli, it does not exceed
3–5 cm. of water. The pressure, then, in the younger *Caudina*, is
1.5–2.5 times as great during contraction as during relaxation.

3. ACTIVITY OF POLIAN VESICLE

According to HEYDE (1922), the water vascular system is conti-
nuous with the tentacular cavities and thus assists greatly in expanding
as well as contracting the tentacles.

We know that all the tentacles do not project at once but usually
one after the other. The same is true for the branches of each
tentacle. Although the entrance of the fluid into the tentacles must
be due to the pressure of the fluid contained in the water vascular
canal from increased contraction of the latter, whether the entire

system of the water vascular canal contracts at once or in part is not clear, though the latter seems more probable. At any rate, whenever a tentacle begins to contract, we find that the fluid in it is first driven back gradually into the ampulla.

The contraction of the polian vesicle produces a current and sends the fluid directly into the radial canal. So that the pressure of the radial canal may be determined from the pressure in the polian vesicle, which value is practically identical with that given by the ampulla, though in this case the tentacular ampulla may affect the result slightly. However, if the polian vesicle is pinched with a forceps, we find practically no change of the pressure in the tentacular ampulla and thus the reciprocal relation between the fluid of the polian vesicle and the tentacular fluid seems smaller than one would anticipate.

4 THE PERIVISCERAL CIRCULATION.

The perivisceral cavity of *Caudina*, like that of other Holothurians, is spacious, and extends from the oral disk to the posterior tip of the body. The fluid is largely sea water (OKAZAKI and KOIZUMI 1927) and contains numerous amoebocytes (CUÉNOT 1891) which are also designated as haemoglobin containing red blood corpuscles, wandering cells (DURHAM 1892), and several other kinds of corpuscles (GEROULD 1896, KAWAMOTO 1927, SIVICKIS and DOMANTAY 1928). Most of all these corpuscles are found in the connective tissue layer of the body wall, vascular system, and tentacular fluid. It is a matter of general knowledge that the perivisceral fluid is in constant communication with the vascular system through the stone canal and the madreporite and therefore the perivisceral fluid may be identical with that of the water vascular system. Moreover some of the haemal system communicates with the water ring canal (KAWAMOTO 1927, SIVICKIS and DOMANTAY 1928), therefore blood and perivisceral fluid are afforded a chance to mix with each other.

The movement of body fluid has been studied hitherto mainly in connection with the ciliary movement. GEROULD in 1896, found that the peritoneal surface of the body cavity of *Caudina* consists of a pavement endothelium which is provided with vibrating cilia, but nothing was stated as to the phenomena of perivisceral circulation.

According to HEYDE (1922), in Echinoderms the perivisceral fluid is stagnant and without current. On the contrary in *Caudina*, we can observe a current of definite speed which maintains a constant circulation within the perivisceral cavity.

The course of the perivisceral circulation is exclusively determined by the movement of cilia, similar to that produced by most other lower forms. MEYER (1929) first demonstrated the important rôle played by the cilia of body walls in *Tomopteris* (Annelid) and reported that the direction of the perivisceral circulation was absolutely constant, 1. e., the direction of the effective beat is the same for all the cilia of one side. On the opposite side (ventral and dorsal) the direction is opposite. The current of the coelomic fluid runs forward on the ventral side of the trunk, and runs backwards on the dorsal side.

In the case of *Caudina*, with which I have experimented, a current may be directed antero-posteriorly along near the body wall or outside of the center of the body axis, and in the region where the tail begins most of the current takes a reversed or posterio-anterior course, though the remaining current proceeds without changing its original antero-posterior course into the tail and, only at the tip end of it, takes the reversed or postero-anterior course along the center of the tail and enters into the body proper. This current runs along the center, uniting with the main upward current, mentioned above, and finally reaches the anterior tip of the body cavity. The general course of the current may be understood better from the following diagram.

Dorsal mesentery

Ventral mesentery   Lateral mesentery
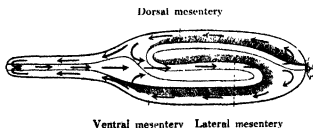
Fig. 1. Diagramatic view of the general course of currents of the perivisceral fluid in *Caudina chilensis*, showing the perivisceral cavity of the body.

In studying these phenomena of the perivisceral circulation both

adult and younger specimens were employed.

Since the body of the adult *Caudina* is opaque and the circulation can not be observed directly, I have determined it indirectly by the colorimetric method. This colorimetric method is (1) to inject some sea water in which a small amount of $K_4Fe(CN)_6$ was dissolved to one part of the perivisceral cavity, and then (2) to test the perivisceral fluid which was drawn from another part of the body with $FeCl_3$.

By this method we could determine not only the direction of the current but how far and how fast it was for any given time. For instance, if we injected a $K_4Fe(CN)_6$ mixture in the anterior part of the perivisceral cavity, we could not detect it with $FeCl_3$ at the place where the injection was made even if it was examined within a second or two after injection, but we invariably detected it in the posterior part of the trunk. On the other hand, the mixture which was injected in the posterior part could be found after some seconds in the anterior part of the cavity, but not at the region where it was injected.

From a number of observations carried out on many specimens it became certain that the perivisceral fluid is circulating antero-posteriorly along the periphery and then reverses its course; that is, postero-anteriorly along the central axis of the body cavity. But such a course of the current as just stated is often disturbed, especially at the time when the *Caudina* is dug out from the sand, owing to a sudden contraction of the body wall, and especially when such contraction occurs irregularly in different parts of the body. Some of the causes which temporarily disturb the current of the perivisceral fluid are enumerated below:

1) Contraction of the body wall.
2) Movement of some ampula of the tentacles.
3) Periodic in- and exhalation of the respiratory tree or water lung.
4) Undulatory movement of the mesentery as well as of respiratory trees, which may further enhance the disturbance of the current motion.

These four factors just stated often produce a complete reversal of the normal course of the current, or force it sidewardly for a few seconds, though such disturbances are soon subsided and the current resumes its normal, original course. These phenomena can easily be

observed in the younger *Caudina* (about 5 mm. in length), in which
the body wall is thinner and transparent, and thus not only the normal
course of the current but the reaction of color is directly observable
under the microscope.

I may add also that in the younger *Caudina*, not only the move-
ment of corpuscles in the perivisceral fluid, but movement of the
respiratory trees and of the tentacular ampullae etc. can clearly be
observed. The results of observations made on the younger *Caudina*
under the microscope accord entirely with the results indirectly obtained
by the method of injection $K_4Fe(CN)_6$; that is, the main course of
the current of the perivisceral fluid is antero-posterior along the peri-
phery of the cavity and posterio-anterior along the centre of the body
cavity.

Some notion as to the velocity of the current of the perivisceral
fluid is very easily obtained when one determines the speed of the
movement of the corpuscles through the skin of the young *Caudina*
(5-15 mm. in length) under the microscope. By the aid of the micro-
meter, the velocity of the corpuscle flow at 15°-18°C sea water, from
several young *Caudina* (5-15 mm. in body length) was determined and
the results are given below.

Length of body.(mm)	Temperature of sea water. (C°)	Velocity per second (mm.)
5	15	13
„	„	12
6	„	10
„	„	14
„	„	13
„	16	15
„	18	11
7	15	10
„	„	13
„	16	14
8	15	15
„	„	12
„	16	13
„	17	15
10	15	10

Length of body. (mm.)	Temperature of sea water (C°)	Velocity per second. (mm.)
10	17	12
„	„	10
„	18	13
„	„	14
„	„	15
„	„	16
15	. 15	12
„	„	14
„	16	13
„	17	13
„	„	15
Average		13

These figures indicate that the individual variations are rather small and that in *Caudina*, ranging in body length range from 5 to 15 mm., the velocities of corpuscles are from 10-16 mm. per second, or in the average 13 mm. per second, at the given temperature. These direct measurements based on the movement of corpuscles are, however, close approximations only since the fluid in reality runs somewhat more quickly than the corpuscles as tested by the colorimetric method.

The velocity appears to depend very largely upon internal pressure produced by the contraction of the body wall. The following figures were obtained from young *Caudina* (18 mm. in length) at 19°C sea water:

Internal pressure in cm water		3	4	5	6
Velocity per second (mm.) I		14	17	18	18
II		16	18	20	21
III		17	21	22	22
Average		16	19	20	20

These relations show that the increased pressure of the cavity during contraction causes also increase of the velocity of the current and, indeed, when the pressure of the cavity is doubled, the velocity of the current becomes 1.3 times its initial value.

In the adult forms, a given pressure produces greater velocity than in the younger specimen, due probably to a greater contractility of the body wall. The facts just mentioned are shown in the following figures. The data were obtained by the colorimetric method of $K_4Fe(CN)_6$ injection.

Internal pressure in cm. water		18	30	40
Velocity per second (mm)	I	14	20	27
	II	12	28	32
	III	15	30	30
Average		14	26	30

That is, when the pressure is doubled, the velocity becomes also nearly double its initial value (by graphic interpolation).

5 TENTACULAR FLUID

GEROULD (1896) observed a current of water setting upwards from the basal region of the tentacular canal and has indicated the general course of tentacular circulation in the figure. However there still remain many interesting problems connected with the course of the current in this important organ.

The tentacular canal is lined with flat ciliated cells. By the movement of these cilia the tentacular fluid circulates, but in a definite direction. The tentacular circulation of *Caudina* has no connection with that of the perivisceral fluid and thus it has its own velocity, though variable under variable conditions. When the tentacle contracts quickly, the velocity of circulation quickly increases but when it stretches, the velocity is slowed.

The direction of the circulation along the inner and outer sides of the tentacular canal (with respect to the center of the body) is opposite to one another, that is, the current along the inner side runs upwards, while that along the outer side runs backwards. My observation on the tentacular circulation was chiefly made on the younger animal in which the tentacular canal is seen clearly through the thin transparent wall. The tentacle with bifurcated branches is the most convenient stage to work with although even in the tetrafurcated stage the cir-

culation of the fluid may easily be observed through the transparent wall.

GEROULD's illustration (1896) shows that the current circulates from the base of the tentacular canal to the tip of one branch then to the tip of another branch along one side and finally runs back to the base again along the opposite side. My own observation reveals, however, that in the bifurcated stage tentacles a current runs along the inner side of the tentacular canal but at the base of division of the branches it divides into two streams and each enters its respective branch. The fluid thus entered proceeds into the tip where it runs back along the opposite side of the canal
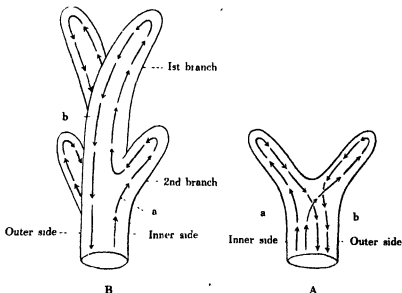
Fig. 2. Diagramatic view of the general course of currents of the tentacular fluid in *Caudina chilensis*. A. Bifurcated tentacle with its canal. B. Tetrafurcated tentacle with its canal. The courses of the current are:
  a, ascending current along the inner side,
  b, descending current along the opposite, outer side.

In the tetrafurcated tentacular stage, the tentacular circulation is some what complex than that of the bifurcated tentacular stage. The

fluid which flows upwards along one side of the canal enters first into the lower 2nd branch and then into the 1st branch. The general course of the current, inhalent and exhalent, is the same as in the former bifurcated stage. From the above it is evident that the general course of circulation of the branches appears first along the side facing towards the mouth of the tentacle, then returns into the main canal through the opposite side and thus seems to avoid a mixing of the upward and downward currents. Such a definite course of circulation would be established by the ciliary movement.

When the tentacle contracts the fluid within the branches is emptied first into the tentacular canal, which in turn empties it into the ampulla in association with the retraction of the entire tentacle. Even if the contraction occurs in only one branch, the fluid in the opposite branch appears to circulate with the same velocity. In fact, the tentacular fluid is always in motion, even when the tentacles remain quiescent. The current is noticed even when the *Caudina* is under the influence of anaesthetics to such an extent that weak mechanical or electrical stimuli produce no response in the tentacles.

SUMMARY.

1. The circulations of the perivisceral fluid and the tentacular fluid in *Caudina chilensis* were examined.

2. The current of the perivisceral fluid progresses antero-posteriorly close along the body wall, and returns postero-anteriorly along the centre of the body cavity.

3. The current of the tentacular fluid runs upwards along the inner side of the canal, that is, that facing towards the oral opening of the branches of the tentacle, and then returns into the tentacular ampulla along the opposite side of the tentacular canal.

4. With respect to the pressure of the perivisceral fluid, it exhibits considerable variations under varied conditions, and shows in the adult *Caudina* about 15 cm/water at the resting time, while during contraction, about 40 cm/water. The pressure of adult animals is much higher than that of younger animals in a similar physiological state.

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Chromosomenmorphologie von *Rumex Acetosa*.

Von

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(Mit 3 Text-figuren.)

Zytologische Untersuchungen von *Rumex Acetosa* wurden schon von Kihara und Ono (1923, 1925), Sinotô (1924), Ono und Shimotomai (1928) und Ono (1928) ausgeführt. Durch diese Untersuchungen sind die Beziehungen zwischen der Sexualität und der Chromosomenformel dieser Pflanze als folgende festgestellt worden:

Sexualität	Chromosomenformel	Autor
♀	$14 = 2X + 12a$	Kihara und Ono (1923, 1925),
♂	$15 = X + 2Y + 12a$	Sinotô (1924)
♀	$21 = 3X + 18a$	Ono (1928)
♀	$22 = 2X + 2Y + 18a$	Ono und Shimotomai (1928)
♂	$29 = 3X + 2Y + 24a$	Ono und Shimotomai (1928)

Seit 1926 habe ich etwa hundert Intersexualpflanzen von *R. Acetosa* in der Umgebung von Sendai gesammelt. Die Chromosomensätze dieser Individuen waren nach meiner bisherigen Untersuchung meistens triploid und nur in einem Falle tetraploid. Vor kurzem habe ich ausserdem noch einige diploide Intersexualpflanzen gefunden. Früher richteten wir unsere Aufmerksamkeit hauptsächlich auf die Chromosomenzahlen, weil die Analyse der einzelnen Chromosomen fast unmöglich schien. Zur genaueren Erkenntnis der Geschlechtsverhältnisse ist aber die Chromosomenmorphologie von grossem Wert. Darum habe ich bei vorliegender Untersuchung diesem Punkt grosse Aufmerksamkeit gewidmet und zur Fixierung der jungen Sprosse Kiharas Abkühlungs- (Kihara, 1927) und für die der Wurzelspitzen die Chloralisierungsmethode (Sakamura, 1920, Kagawa, 1929) angewendet. Als Fixierungsflüssigkeit habe ich Carnoys Gemisch für die Sprosse und das Flemmings für die Wurzeln gewählt. Diese beiden Methoden lieferten immer sehr gute Resultate. Hier teile ich meine vorläufigen Ergebnisse kurz mit.

DIPLOIDE PFLANZE.

Hier möchte ich zuerst die Chromosomenmerkmale der normalen männlichen und weiblichen Pflanzen genauer beschreiben.

Die männliche Pflanze enthält $15(=X+2Y+12a)$ diploide Chromosomen. Fig 1 und 8 zeigen die somatischen Kernplatten aus der Archespor- und Wurzelteilen dieser Pflanze. Wie man aus den Abbildungen sieht, sind das X-Chromosom und die zwei Y-Chromosomen im Vergleich zu den übrigen Autosomen in Länge und Form charakteristisch. Das X ist am längsten und besitzt in der Mitte ein Gelenk od. eine Einschnürung, die eine Insertionsstelle der Zugfasern ist. Die zwei Y sind auch durch ihren gleich od. ungleich zweischenkeligen Bau charakteristisch, aber sie sind immer kürzer als X. In der Regel hat das eine (y_1) das Gelenk fast in der Mitte, und das andere (y_2) hat es etwas entfernt. Die Länge des y_1-Chromosoms ist im allgemeinen etwas grösser als die des y_2. Die übrigen 12 Autosomen bilden gewöhnlich gerade Stäbchen, bei denen an einem Ende infolge der Einschnürung ein Köpfchen sichtbar ist. Es ist aber schwer, die einzelnen Autosomen zu unterscheiden. Öfters finden wir Trabanten an einem relativ langen Paar von Autosomen. — Sie können auch öfters beim Weibchen beobachtet werden. — Unter Umständen können sie jedoch gar nicht gesehen werden.

Die weibliche Pflanze enthält $14(=2X+12a)$ somatische Chromosomen. Wie die Figuren (Fig. 3 und 7) zeigen. können wir klar 2 lange, zweischenkelige X und 12 gerade Autosomen mit je einem Köpfchen an ihrem proximalen Ende feststellen. Die Gestalt und das Längenverhältnis eines vollständigen Chromosomensatzes der männlichen und der weiblichen Pflanze sind in Fig. 3 und 4 dargestellt.

Wie schon erwähnt, habe ich neuerdings einige 15-chromosomige Intersexualpflanzen gefunden. Der Intersexualitätsgrad dieser Pflanzen ist aber sehr niedrig[1]. Die somatische Kernplatte solcher Pflanzen ist in Fig. 2 a, 5 a, 6 a und 9 abgebildet. Der Chromosomensatz ist sehr ähnlich dem der männlichen Pflanze. Ein Punkt ist aber ganz sonderbar. Es ist nämlich immer ein verhältnismässig kürzeres, zweischen-

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[1] Die Intersexualpflanzen von *R. Acetosa* sind meistens andromonözisch od. trimonözisch. Ich habe die Grade der Intersexualität begrifflich durch die Berechnung der Prozentsätze ihrer bisexuellen Blüten bestimmt (ONO, 1930).
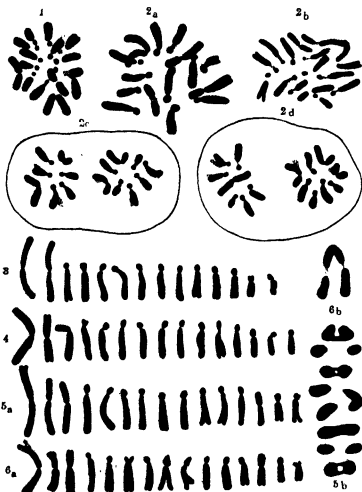
Rumex Acetosa. Fig 1 Diploid r Chromosomenbestand einer Archespor zelle des normalen Männchens (15 = X + 2Y + 12a) Fig. 2 a-d Chromosomenbestand einer 2 n Intersexualpflanze Nr 0 (15 = X + 2Y + a' + 11 a) Die Figuren beziehen sich auf ein und dieselbe Pflanze. Fig 2 a Polplatte aus der Tapetenzelle. Fig. 2 b Polplatte aus der Archesporzelle   Fig. 2 c, d Homöotypische Kernplatten. (X + a' + 5 a) + (2Y + 6 a) (X + 6 a) + (2Y + a' + 5 a). Fig 3–6 Verhalten der Chromosomenbestände normaler und intersexueller 2 n Pflanzen Die Chromosomen sind ihrer Grösse nach geordnet Fig. 3 Normales Weibchen (14 = 2X + 12 a) Fig. 4. Normales Männchen (15 = X + 2Y + 12 a). Fig. 5 a. 2 n Intersexualpflanze Nr 102 (15 = X + 2Y + a' + 11 a) Fig. 5 b Bivalenten in einer Pollenmutterzelle aus derselben Pflanze (6 ll + YXY). Fig 6 a 2 n Intersexualpflanze Nr. 77 (15 = X + 2Y + a' + 11 a) Fig 6 b Ein dreiteiliges Chromosom (YXY) in derselben Pflanze × ca 2000
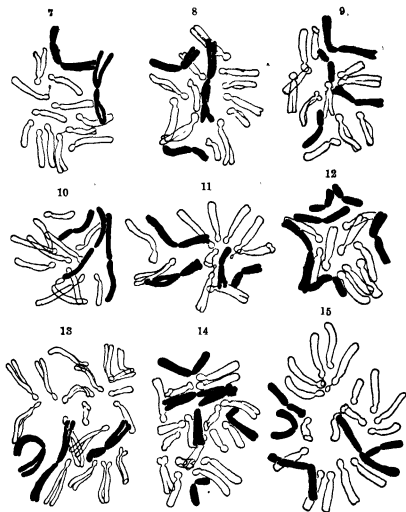
Rumex Acetosa. Fig. 7–21. Diploide Chromosomenbestände der verschieden-
en Individuen X-, Y- und a'-Chromosomen sind schwarz gezeichnet. Fig. 7.
Normales Weibchen (14 = 2X + 12 a)   Fig. 8. Normales Männchen (15 = X + 2Y
+ 12 a)   Fig. 9 2 n Intersexualpflanze Nr 71 (15 = X + 2Y + a' + 11 a). Fig. 10 u.
11. 2 n Weibchen kombinativ mit anderen Chromosomenbeständen. Fig. 10.
14 = 2X + Y + 11 a. Fig. 11. 14 = 2X + 2 a' + 10 a.   Fig. 12 Nachkomme einer 3 n
Intersexualpflanze (15 = X + 3Y + 2 a' + 9 a). Fig. 13 3 n Weibchen (21 = 3X + 18 a).
Fig. 14, 15. 3 n Intersexualpflanze mit einem a'-Chromosom (22 = 2X + 2Y + a'
+ 17 a). × ca. 2000.

keliges Autosom bei diesem Falle bemerkbar. Dieses Autosom hat eine
Einschnürung an einer Stelle nicht so fern von der Mitte und ist
ungefähr so lang wie ein Schenkel des X-Chromosoms. Ich bezeichne
dieses Autosom hier mit dem Buchstaben a' zur Unterscheidung von
den übrigen gewöhnlichen Autosomen (a). Dann ist die Chromosomen-
formel dieser Pflanzen, wie folgt: $15 = X + 2Y + a' + 11$ a. Das a'-
Chromosom habe ich bei der Kernteilung in den Archespor-, Tapeten-
und Wurzelzellen und besonders klar bei der homöotypischen Teilung
der Pollenmutterzellen gefunden (Fig. 2 a–d). Bei der heterotypischen
Teilung liesse sich ein heteromorphes Paar erwarten, welches das a'-
Chromosom mit einem gewöhnlichen Autosom gebildet hat. Aber das
kann in der Wirklichkeit nicht beobachtet werden. Jedoch werden
vier Arten von homöotypischen Kernplatten, deren Chromosomenformel
$(X + a' + 5$ a$)$, $(2Y + 6$ a$)$, $(X + 6$ a$)$ und $(2Y + a' + 5$ a$)$ sein sollen, in
den Pollenmutterzellen in fast gleicher Häufigkeit angetroffen (Fig.
2 c, 2 d).

In diesem Jahre (1929) habe ich einige Kreuzungen zwischen
diesen Pflanzen und dem normalen Weibchen vorgenommen. Das
karyologische Verhalten dieser Bastarde werde ich später mitteilen.
Im Freien habe ich einige eigentümliche diploide weibliche Indivi-
duen gefunden, deren Chromosomenformeln $14 = 2X + a' + 11$ a, $14 =
2X + 2$ a$' + 10$ a (Fig. 11) oder $14 = 2X + Y + 11$ a (Fig. 10) sind.

TRIPLOIDE PFLANZE

Die frühere Mitteilung von ONO und SHIMOTOMAI (1928) hat ge-
zeigt, dass die im Freien gefundenen Intersexualpflanzen meistens
triploid $(22 = 2X + 2Y + 18$ a$)$ sind. Später habe ich (ONO, 1928) auch
eine triploide $(21 = 3X + 18$ a$)$ und weibliche Pflanze gefunden. Zu
jener Zeit war unsere Analysierung der einzelnen Chromosomen-
elemente dieser beiden Arten der triploiden Pflanzen, besonders inbezug
auf die Autosomen ungenügend. Deshalb habe ich sie an den neu
hergestellten Präparaten nochmals ausgeführt.

Beim 21-chromosomigen Individuum konnte ich 3 lange, zweischen-
kelige X-Chromosomen und 18 gerade Autosomen mit je einem Köpf-
chen finden. Aber das oben erwähnte, zweischenkelige a'-Chromosom
wurde niemals beobachtet (Fig. 13). Dieses Resultat stimmt also mit

meiner früheren Formel 21 = 3X + 18 a überein.

Mein diesmaliges Ergebnis bei den 22-chromosomigen Intersexual-
pflanzen ist aber zum Teil etwas anders als das frühere. Bisher habe
ich 28 Individuen untersucht. Daraus ziehe ich folgende Formeln:
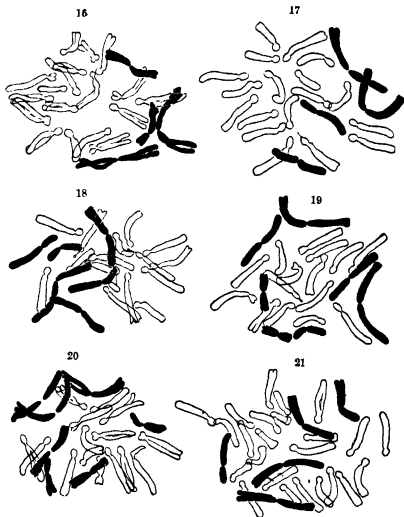
Rumex Acetosa. Fig. 16, 17. Normale 3 n Intersexualpflanzen (22 = 2X + 2Y
+ 18 a)   Fig. 18–20. Kombinativ anormale 3 n Intersexualpflanzen. Fig. 18. 22 =
2X + 3Y + a' + 16 a. Fig 19, 20. 22 = 2X + 2Y + 3 a' + 15 a. Fig. 21. 4 n Intersexual-
pflanze (29 = 3X + 2Y + 24 a). × ca. 2000.

$22 = 2X + 2Y + 18$ a (18 Individuen) Fig. 16 u. 17

$22 = 2X + 2Y + a' + 17$ a (6 Individuen) Fig. 14 u. 15

Der Intersexualitätsgrad der Individuen mit dem a'-Chromosom ist im allgemeinen stärker als der der es nicht besitzenden Individuen.

Unter den 22-chromosomigen Intersexualindividuen habe ich ausserdem noch 4 andere Pflanzen mit abweichenden Chromosomenelementen gefunden. Die Chromosomenformeln dieser Pflanzen sind vielleicht folgende:

$22 = 2X + 3Y + a' + 16$ a (2 Individuen) Fig. 18

$22 = 2X + 2Y + 3$ a' $+ 15$ a (2 Individuen) Fig. 19 u. 20

Die Chromosomenformel des in Fig. 20 gezeigten Individuums ist etwas fraglich, weil die Unterscheidung des Chromosoms X und Y allerdings oft beträchtliche Schwierigkeiten macht. Diese Pflanzen zeigen aber verhältnismässig starke Intersexualität.

TETRAPLOIDE PFLANZE

Hierher gehört bisher nur ein Individuum. Die Chromosomenformel dieses Individuums ist schon als $29 = 3X + 2Y + 24$ a angegeben worden. Die diesmalige Untersuchung hat auch dasselbe Resultat gebracht. Und unter den Autosomen wurden nie zweischenkelige a'-Chromosomen gefunden (Fig. 21). Diese Pflanze zeigt mittlere Intersexualität.

NACHKOMMEN DER TRIPLOIDEN UND TETRAPLOIDEN

Einige der oben erwähnten triploiden und tetraploiden Pflanzen sind öfters fertil. Ich habe allein im Jahre 1929 etwa 200 Nachkommen gewonnen. Einige von ihnen habe ich zytologisch untersucht. Die festgestellten Chromosomenformeln sind folgende: $15 = X + 3Y + 2$ a' $+ 9$ a (Fig. 12), $15 = 2X + 13$ a, $16 = X + 2Y + 13$ a und $20 = 2X + Y + 17$ a. Weitere Ergebnisse der Untersuchungen in dieser Richtung werden in der Folge ausführlich berichtet werden.

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Embryological Studies on Sargassum.

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(With 13 Text-figures)

INTRODUCTION.

Embryological studies on Sargassum, Turbinaria (?), Cystophyllum and Coccophora, all members of Fucaceae, have recently been carried out by TAHARA (1928, '29) and OKABE (1929). The results of these authors appear to throw some light on the systematic position of these algae.

Among the genera above mentioned, Sargassum is the largest one. According to YENDO's monograph on Japanese Fucaceae (1907) we have 41 species of Sargassum on our coast. To our regret, however, hitherto only two species, S. Horneri and S. Thunbergii have been investigated embryologically. So, at the suggestion of Prof. TAHARA further studies in this line on a fairly large number of species of Sargassum have been undertaken since the spring of last year.

MATERIALS AND METHOD.

As is well known, the liberation of sexual cells in Sargassum occurs simultaneously and periodically and, in most species, usually in the spring tide. The season of the ripening of sexual cells is different in each species, so I visited the Marine Biological Stations at Misaki and Asamushi several times from March to July to collect the materials for the study. The dates of oogonium liberations in the different species are shown in the following table:

TABLE I.

Specific name	Nom. Jap.	Date of Oogonium liberation	Locality
Sargassum *Horneri* AG.	Aka-moku	March 16th	Misaki
,, *enerve* AG.	Hon-dawara	March 16th and May 15th	,,
,, *Kjellmanianum* YENDO	Hahaki-moku	March 18th and May 10th	,,
,, *tortile* AG.	Yore-moku	May 9th	,,
,, *piluliferum* AG.	Mame-tawara	May 12th	,,
,, *hemiphyllum* AG..	Iso-moku	May 13th	,,
,, *nigrifolium* AG.	Narasa-mo	May 13th	,,
,, *confusum* AG.	Fushisuzi-moku	May 30th	Asamushi
,, *patens* AG.	Yatsumata-moku	June 20th	Misaki
, *Ringgoldianum* HARV	Ooba-moku	June 22th	,,
,, *serratifolium* AG.	Nokogiri-moku	June 24th	,,
,, *micracanthum* (KUTZ)	Toge-moku	July 7th	,,

The days of new and full moon of last year are shown in Table II.

TABLE II.

Month	March	April	May	June	July
New moon	11	10	9	7	7
Full moon	25	24	23	22	22

From these tables, it will be seen that in each species the liberation of sexual cells occurs generally in the spring tide.

To make a study of the embryonal development, in the first place some small branches of those algae which carried many discharged eggs on the surface of their receptacles were collected and cultured in small glass basins filled with natural sea water.

The discharged eggs remain attached to the outer surface of the receptacle for several days and begin to develop there. But before the rhizoid formation begins in the lower extremity of the embryos,

they are detached from the receptacles and fall to the bottom of the glass basin and further development continues in that condition.

For the fixation of the embryos, FLEMMING's weaker solution prepared with sea water was used exclusively, and the microtome sections, generally cut in 5-10 μ, were stained with safranin and light-green.

OBSERVATION

Eggs discharged from the conceptacle are generally ovoid or ellipsoid in shape and are covered with a thick layer of gelatinous substance. The first segmentation-wall runs transversely. The second one is also transverse and cuts a small lense-shaped rhizoid cell in the lower extremity of the embryo.

The further segmentations of this rhizoid-cell are quite interesting and make a significant characteristic of each species. In the present paper particular attention is paid to this point. Detailed description will be given below.

PLANTS INVESTIGATED

I. *Sargassum hemiphyllum* Ag.

This plant is strictly dioeceous and receptacles of both sexes have a similar external appearance. They are small and cylindrical, generally measuring about 2 mm. long, and ripen at Misaki about the middle of May. The discharged eggs measure 125 μ long and 105 μ wide.

The first two segmentation walls in the rhizoid cell run vertically through the center of the cell and are perpendicular to each other. But the third goes quite irregularly. Some are parallel with the former ones, while some are oblique than through the center, just as described in *S. Thunbergii* by TAHARA (1929) (Text-fig. 1). The rhizoid cell in the eight-cell stage is about 43 μ in diameter. Later a group of eight rhizoids is developed, one rhizoid being developed in each cell.
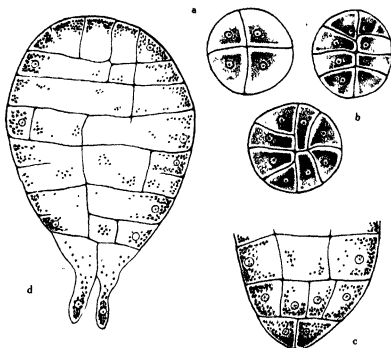
Text-fig. 1 *Sargassum hemiphyllum.* a, Four cell stage of the rhizoid cell. b, Eight cell stage of the same   c, Longitudinal section of the rhizoidal portion of an embryo   d, The same in a still later stage   ×420
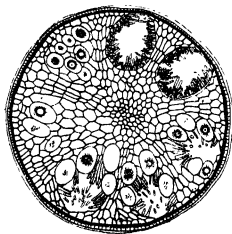
Text-fig. 2. Cross section of a receptacle of *Sargassum Kjellmanianum.* ×72.

II   *Sargassum Kjellmanianum* YENDO.

This plant is typically monoeceous. The male and female conceptacles are contained in the same cylindrical receptacle, which measures about 10–12 mm. long (Text-fig. 2). The receptacles ripen at Misaki generally from the middle of March to the latter part of April. The discharged eggs mea-

sure 139 μ long and 97 μ wide.

The manner of embryonal development of this species is just the same as that of *S. hemiphyllum*. The rhizoid cell in the eight-cell stage is about 63 μ in diameter, that is, slightly larger than that of the former species. Rarely, the rhizoid cell of this plant is divided into nine or ten cells (Text-fig. 3. d), a feature perhaps suggesting that this species is higher in systematic position than the former one.
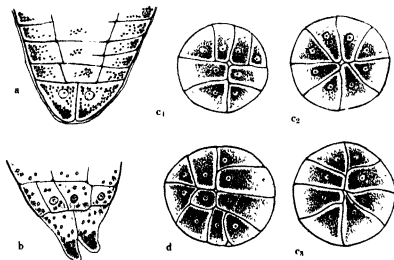
Text-fig. 3 *Sargassum Kjellmanianum* a, Longitudinal section of the rhizoidal portion of an embryo b, The same in a still later stage c_1, c_2, c_3, Eight cell stage of the roizoid cell. d, Rhizoid cell divided into 10 cells. ×420

III. *Sargassum confusum* AG.

Dioeceous. Receptacles cylindrical, its entire length being 10–15 mm. in the male, and 7–10 mm. in the female. Germ-cell liberations occur at Asamushi from the latter part of May to the middle of June. The discharged eggs measure 210 μ long and 140 μ wide.

The rhizoid cell is divided into eight (rarely nine or ten) cells, as in *S. Kjellmanianum* (Text-fig. 4), and measures, in this stage, about 60 μ in diameter, that is, about the same as that of the former species.

IV. *Sargassum enerve* AG. ·

Dioeceous. The male receptacles are slender, often measuring 18 mm. long, while the female ones are fatty, 13–15 mm. long. They
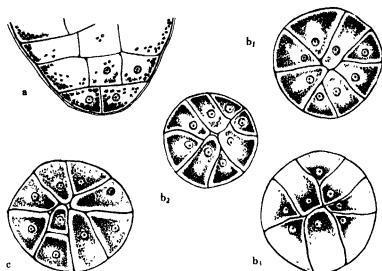
Text-fig 4. *Sargassum confusum*   a, Longitudinal section of the rhizoidal portion of an embryo b_1, b_2, b_3, Eight cell stage of the rhizoid cell   c, Rhizoid cell divided into nine cells   × 420
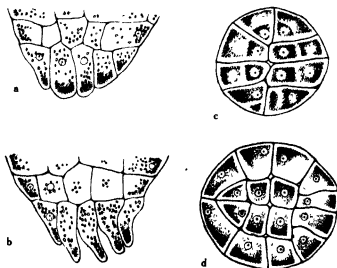
Text-fig. 5 *Sargassum enerve*. a, Longitudinal section of the rhizoidal portion in the sixteen cell stage. b, The same in a still later stage. c, Eight cell stage of the rhizoid cell. d, Sixteen cell stage of the same. ×420.

ripen at Misaki from the beginning of January to the latter part of April. The discharged eggs measure 250 μ long and 235 μ wide, about twice as large as the ones of *S. hemiphyllum.*

The rhizoid cell division of this species differs from that in the foregoing ones. At first it divides into eight cells, without unusual up to this stage. But then each of these eight cells divides once more and sixteen cells are produced, as shown in Fig. 5, and the rhizoid formation begins in this stage. The rhizoid cell of the 16-cell stage measures about 75 μ in diameter.

Later, a group of sixteen rhizoids is developed at one extremity of the embryo; rhizoids which have no direct relation to the rhizoid cell can not been, at least in the early stage of development.

V. *Sargassum piluliferum* Ag.

Dioeceous. Receptacles small, cylindrical, measuring 3–8 mm. long; the male ones slightly longer than the female. Ripen at Misaki about May The rhizoid cell is divided into sixteen cells as in the former one and measures, in this stage, about 72 μ in diameter.

The fourth division of the rhizoid cell of this species is sometimes omitted in 3 or 4 cells, thus producing only 12 or 13 cells in the final stage (Text-fig. 6. b, c)
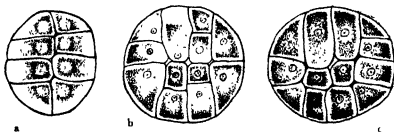
Text-fig. 6. *Sargassum piluliferum.* a, Eight cell stage of the rhizoid cell. b, c, Rhizoid cell divided into only twelve and thirteen cells. ×420.

VI. *Sargassum patens* Ag.

Dioeceous. Receptacles linear, simple or sometimes divided, measuring 5–7 mm. long. Ripen at Misaki about June. The discharged eggs measure 218 μ long, 177 μ wide. The rhizoid cell is divided into sixteen cells and measures, in this stage, 67 μ in diameter.

The fourth division of the rhizoid cell is omitted sometimes in ¦3
or 4 cells, as in the former species (Text-fig. 7. d₁, d₂). The fourth
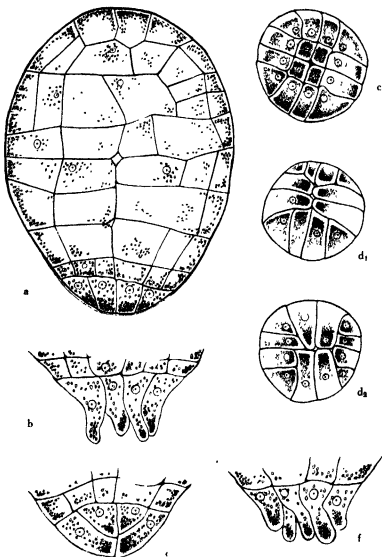
Text-fig. 7. *Sargassum patens.* a, Longitudinal section of an embryo. b,
The rhizoidal portion in a still later stage. c, Sixteen cell stage of the rhizoid
cell. d₁, d₂, Rhizoid cell divided into only twelve cells. e, Two-storied cell
arrangement in the rhizoidal portion. f, The same in a still later stage. × 420.

division is, however, occasionally carried out parallel to the outer
surface, forming, as a result, a two-storied cell arrangement in the
rhizoidal portion, as seen in *Cystophyllum sisymbrioides* (OKABE 1929)
(Text-fig. 7. e).

VII. *Sargassum Ringgoldianum* HARV.

Dioeceous. Receptacles differ greatly in external shape according
to sex. The male receptacles are linear-spathulate, measuring 5 cm.
long and 7 mm. wide, while the female ones are compressed siliquae-
form, measuring 11 mm. long and 3 mm. wide. Germ-cell liberations
occur at Misaki from the latter part of June to the middle of July.
The discharged eggs measure about 222 μ long, 135 μ wide. The
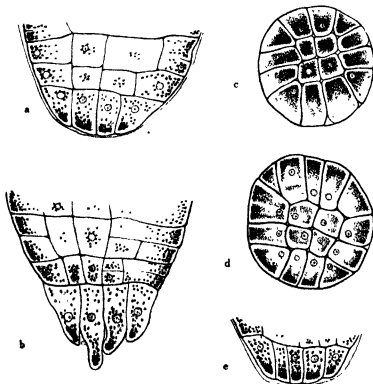
Text-fig. 8. *Sargassum Ringgoldianum* a, e, Longitudinal section of the
rhizoidal portion. b, The same in a still later stage. c, Sixteen cell stage of
the rhizoid cell. d, Rhizoid cell divided into 17 cells. ×420.

rhizoid cell is divided into sixteen cells and measures, in this stage, about 75 μ in diameter (Text-fig. 8).

It is noteworthy that in this species one or two of the sixteen cells are rarely divided once more and 17 or 18 cells are produced, as shown in Fig. 8, d.

VIII.   *Sargassum nigrifolium* YENDO.

Dioeceous.   Receptacles spathulate or subcuneate, with a few dentations at the apex, often measuring 7 mm. long and 5 mm. wide. They ripen at Misaki about May.   The discharged eggs measure 264 μ long and 236 μ wide.

Unfortunately, owing to the failure of the culture, only a very few embryos were available and the exact manner of cell division of the rhizoid cell could not be ascertained in this plant. But I suppose it may be the same as found in *S. enerve*, for about 16 rhizoids were counted in the lower extremity of the embryo.

IX.   *Sargassum serratifolium* AG.

Dioeceous.   Receptacles complanated, spathulate-clavate, often measuring 10–15 mm. long.   They ripen at Misaki in June.   The discharged eggs measure 275 μ long and 202 μ wide. The rhizoid cell is divided into sixteen cells and measures, in this stage, about
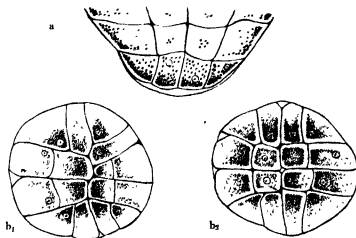
Text-fig. 9. *Sargassum serratifolium*. a, Longitudinal section of the rhizoidal portion b_1, b_2, Sixteen cell stage of the rhizoid cell. ×420.

90 μ in diameter (Text-fig. 9).

X. *Sargassum tortile* AG.

Dioeceous. Receptacles linear-spathulate, often measuring 10–15 mm. long and ripening at Misaki from April to May. The discharged eggs measure 333 μ long and 236 μ wide. The rhizoid cell is divided also into 16 cells and measures, in this stage, 100 μ in diameter (Text-fig. 10).
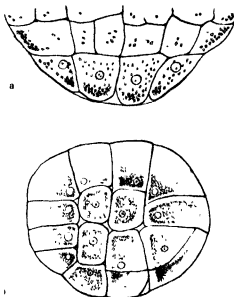
XI. *Sargassum micracanthum* (KÜTZ.)

Dioeceous. Receptacles obovate, the apical margin minutely toothed. They ripen at Misaki from June to July. The egg of this plant is the largest in *Sargassum*, often measuring 384 μ long and 275 μ wide.

When I went to Misaki, it was too late to collect sufficient material for observation and I could not examine the manner of the rhizoid cell division or the number of rhizoids produced in young embryos.

Text-fig. 10. *Sargassum tortile* a, Longitudinal section of the rhizoidal portion b, Sixteen cell stage of the rhizoid cell. ×420
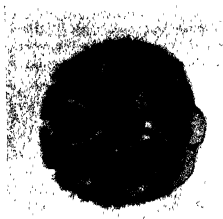
XII. *Sargassum Horneri* AG.

The embryological study of this plant has been carried out already by TAHARA. My own observation at Misaki entirely coincides with his.

The root of this plant, differing from that of other species, is scutellate, irregularly lobed on the margin. Also dioeceous. The large female receptacles are cylindrical, often measuring 20–30 mm. and the male ones are two or three times longer than the female. They ripen at Misaki from January to April. The discharged eggs measure 264 μ long and 198 μ wide.

As Tahara describes, through three successive divisions the rhizoid cell is divided into eight radially' arranged cells and at this stage rhizoid formation begins. At first, eight rhizoids originating from the rhizoid cell are observed, but soon another group of rhizoids developed from the cells adjacent to the rhizoid cells are seen. This group of rhizoids protrudes outward through the central crevice among the group of primary rhizoids (Text-fig. 11).

Text-fig. 11. *Sargassum Horneri*. Rhizoid cell divided into eight radiately arranged cells ×500

DISCUSSION AND CONCLUSION.

From the results above described, the rhizoid formation in *Sargassum* will be divided into three types; namely irregular eight-cell type, sixteen-cell type, and radial eight-cell type.

ı) Irregular eight-cell type.

In this type the rhizoid cell is divided into eight irregularly arranged cells. Later a group of eight rhizoids is developed at one extremity of the young embryo. *S. Thunbergii*, *S. hemiphyllum*, *S. Kjellmanianum* and *S. confusum* belong to this type.

ii) Sixteen-cell type.

In this type the rhizoid cell ıs divided into sixteen irregularly arranged cells. Later a group of sixteen rhizoids is developed at the lower extremity of the young embryo. *S. piluliferum*, *S. patens*, *S. enerve*, *S. Ringgoldianum*, *S. tortile* and *S. serratifolium* belong to this type.

iii) Radial eight-cell type.

In this type the rhizoid cell is divided into eight radially arranged cells. In addition to the eight rhizoids originating from the rhizoid
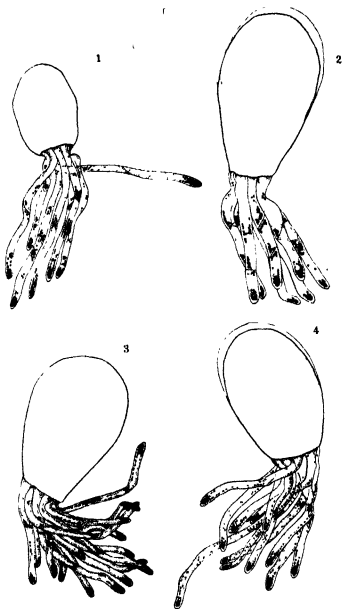
Text-fig. 12. Young embryos having developed their primary rhizoids; 1, *Sargassum hemiphyllum*. 2, *Sargassum confusum* (×196). 3, *Sargassum enerve* (×170). 4, *Sargassum patens* (×252).

cell, another group of rhizoids which are produced from the cells situated just behind the rhizoid cell are seen in the early stage of embryonal development. Only *S. Horneri* belongs to this type.

The plants belonging to the first type, for example *S. hemiphyllum*, *S. Thunbergii* and *S. Kjellmanianum*, generally have a filiform leaf attached to the base of the stipe of the receptacle. It seems to me that this is a feature common to all species of the first type. Further, it may be mentioned that in general the species belonging to the irregular 8-cell type are much smaller in stature and grow on rocks near to the low-tide mark or in slightly deeper places.

S. patens and *S. piluliferum*, which in embryonal rhizoid formation show an intermediate form between the first and the second type, have in the upper portion also linear or filiform receptacular leaves.

S. Horneri, which belongs to the third type, has a number of peculiar characteristics. The peculiar scutellate root, irregularly lobed on the margin (YENDO, OKAMURA 1907, '23), is quite unique. This kind of root can not be found in any other species examined. Furthermore, *S. Horneri* has exceptionally large receptacles, as already mentioned. By some algologists this species is placed under the subgen. *Bacterophycus*, with *S. enerve*, *S. serratifolium* and *S. tortile*. But it seems to me that this alga should be displaced to the other systematic position.

The size of the discharged eggs of each species is different. It seems to me that the algae having larger eggs are higher in systematic position. Data concerning this point are given in the Table III.

As we see from the table, there is a remarkable difference in the size of the eggs. Generally speaking, the eggs of the 16-cell type are much larger than those of the irregular 8-cell type. The average area of the primary rhizoid cell in the former is about twice that of the latter. It may be worth mentioning here that the primary rhizoid cell of the eggs of *Cystophyllum sisymbrioides*, which, as OKABE's recent studies have shown, can be called a 32-cell type, is twice as large as that of the average eggs of the 16-cell type. Thus it is apparent that the number of rhizoids to be developed in the early stage of embryo-formation has a definite relation to the size of the primary rhizoid cell.

TABLE III.

Specific name	Long axis	Short axis	Diameter of rhizoid cell	Type
Sargassum hemiphyllum AG	125 µ	105 µ	48 µ	irreg. 8-cell type
,, *Kjellmanianum* YENDO	139	97	63	,,
,, *confusum* AG	210	140	64	,,
,, *patens* AG	218	177	67	16-cell type
,, *piluliferum* AG	235	110	72	,,
,, *enerve* AG	250	235	75	,,
,, *Ringgoldianum* HARV.	222	153	75	,,
,, *serratifolium* AG	275	202	90	,,
,, *tortile* AG	333	236	100	?
,, *nigrifolium* YENDO	264	236	—	?
,, *Horneri* AG.	264	198	83	radial 8-cell type
,, *micracanthum* (KÜTZ.)	384	275	—	?
Cystophyllum sisymbrioides AG	312	229	120	32-cell type

In my opinion, the species, eggs of which are relatively larger, should be placed in a higher systematic position. Thus the most primitive species in *Sargassum* is to be found in the group of the irregular eight-cell type. *Cystophyllum*, which perhaps may have descended from a *Sargassum* of the 16-cell type, has so far as examined, still larger eggs than any species of *Sargassum* of the 16-cell type
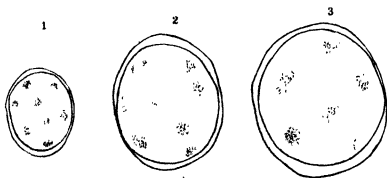
Text-fig 13. Eggs in eight nucleate stage; 1, *Sargassum Kjellmanianum*.
2, *Sargassum enerve*. 3, *Cystophyllum sisymbrioides.*

and should rightly be placed in a higher systematic position than *Sargassum*.

In *Turbinaria* (?) *fusiformis*, which some algologists think should be placed under *Sargassum*, the rhizoid formation (TAHARA '29) resembles closely that of the irregular 8-cell type. So this opinion appears to receive an affirmation from the embryological studies.

SUMMARY.

i) The manner of the rhizoid formation in *Sargassum* is divided into three types; irregular 8-cell type, 16-cell type, and radial 8-cell type.

ii) The rhizoid number in *Sargassum* and *Cystophyllum* has a definite relation to the size of the primary rhizoid cell.

iii) The species having larger eggs are higher in systematic position.

In conclusion, I wish to express my hearty thanks to Professor. M. TAHARA for his valuable suggestions and assistance given me during the progress of this work, and to Dr. K. OKAMURA, Director of the Imperial Fisheries Institute, for his help in the identification of my materials, and to Prof. YATSU, Director of the Misaki Marine Biological Station, through whose kindness many facilities were afforded in the course of my investigations.

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On the Body Temperature of the Earthworm.

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(With 1 Text figure.)

INTRODUCTION.

In these homoiothermic animals is developed a delicate regulatory ability to the changes of an environmental temperature, and by that means their proper body temperature is always constantly maintained regardless of its wider variation.

On the contrary there are so-called poikilothermic animals in which the body temperature fluctuates as the environmental temperature varies. It is usually stated by many authors that in poikilotherms, their body temperature in most cases is more or less higher than that of their environment.

John Hunter (1837) who first measured the body temperature of the earthworm, states that the temperature of the worm was 14.7°C. when that of the environment was 13.3°C. His determination, like that of most other investigators at that period, was made by a mercury thermometer.

Recently Rogers and Lewis (1914 and 1916) carried out a series of observations on the body temperature of earthworms by using the most accurate method of thermometry, the thermoelectric thermometer which was capable of resistering a difference of 0.0042°C., and found that the body temperature of the earthworm approximates closely to that of its environment.

As far as I am aware, most investigators have observed the regulatory ability of the body to the surrounding temperature in very narrow range and indeed even in Roger's work it was determined when the earthworms were subjected to the temperature at between 16.10° and 22.15°C.

It seems therefore highly desirable to have a more adequate

understanding of the physiological nature of poikilotherms, and data on the adjustment of body temperature in a wider range, including critical temperatures compatible with life.

In this point of view, the present investigation was undertaken to determine the ability of the adjustment on body temperature within a wide temperature range, by means of the thermoelement, as will be described in the following pages.

Particular thanks are due to Prof. Dr. S. HATAI for his kind direction in preparation of this work, and also to many others in this Institute who helped me in many ways while the work was progressing.

APPARATUS AND METHOD.

It is a well known fact that a thermal electric current is developed when the junctions of different metal wires of a common circuit are not at the same temperature, and that it is approximatly proportional to the difference of the temperature of the junctions.

This principle was applied first by MALE von NOBILI and MELLONI in 1831 to living animals (e. g. larva, pupa and imago of an Insect).

Since that time more highly sensitive galvanometers provided with pure metallic wires of small diameter, and of small heat capacity came in to use. ROGERS and LEWIS, applying such a method of temperature measurement as just stated to living poikilothermic animals, such as goldfish, salamanders, anadonta and earthworms, were able to detect so minute a temperature difference as two thousandths of a degree of Celsius in contrast with a given surrounding temperature.

I shall briefly describe the method which was employed in my work :

(1) *Thermoelement.* — The thermoelements used were 4-junction thermoelements, and consisted in double silk covered copper wire of No. 36 B. S. and No. 36 B. S. constantan wire.

The constantan wire was insulated by covering it with thin elastic paper (like tracing paper), at first, and then immersing it into melted yellow wax till absorbed well. As soon as the wax was dried, it was varnished with white enamel and hung for a couple of days or more in the corner of the Laboratory room, until it became completely

dried. Both the copper and constantan wires were carefully shellacked. In making the thermoelements I followed the method of WHITE. The two kinds of wires were uncovered for a short distance (about 1 cm.), were twisted together at the ends, and after being polished well with rosin, were dipped into melted solder. All trace of shellack must be completely removed in order to make the ends sensitive even for a very small magnitude of heat.

Four junctions were connected with a common galvanometer circuit, and two junctions were each enclosed in a glass tube whose diameter was just large enough to contain it; that is, one end of a glass tube, 15 cm. long and 8 mm. in diameter, was narrowed by heating on the Bunsen burner to 3 mm. in diameter and about 4 cm. long, and the tip was sealed.

This tube thus stretched was gently curved (about 130°) at the point where the narrowing began, in order to be pushed into the anal or buccal opening of the earthworms conveniently. To this bent tube thus prepared the two wires were sealed and the open end was closed with wax in order to prevent the entrance of any water while manipulating.

The four junctions of the element sealed in the two glass tubes were connected directly to the galvanometer. When the thermoelement was not in use it was kept as much as possible at the same temperature.

I adjusted my apparatus in such a way that a change of one degree corresponded to a deflection of 100 mm. on the scale at a distance of 0.7 m. from the mirror to the scale. Since we can read a half of one mm. in the scale division, we are able to determine the difference of as small an amount as 0.002°C.

This thermal difference of 0.002° corresponds to 0.83 microvolt, which was determined by Type K potentiometer of Leads and Northrup Co.

Since the actual amount of deflection varies slightly daily, it was necessary for me to re-determine its actual value each day.

The parasitic current in the circuit was detected from the amount of deflection from the zero point by attaching the junctions together in the thermostat and if it was too large to effect the result, the observation was suspended until the galvanometer registered the usual zero point.

(2) *Galvanometer.* — The galvanometer used was the table type of D'Alsonval made by Leads and Northrup Co., and the constants of the galvanometer were as follow :

Sensitivity........93 megohms

Resistance........115 ohms

Period .... 7 seconds.

The galvanometer was placed on the stone table so as to be freed from the vibration from without.

The deflection readings were taken with lamp and scale (which divided into 1 mm.), at a distance of 0.7 m. from the mirror of the galvanometer and the scale.

(3) *Thermostat.* — The thermostat was provided with an electric lamp heater, electric thermoregulator and fan and motor. In the thermostat was placed a dish of 18 cm. diameter, for holding the earthworm.

(4) *Procedure of the thermometry.* — It is very simple, indeed, to measure the difference between the body temperature of the earthworm and the environment

The two junctions of the element are immersed into the water of constant temperature in the dish which is later placed in the thermostat, and are allowed to remain until deflection stops. This point of standstill is taken for the zero point of the scale for the experiment. For further control the temperature of water in the dish was also recorded by a certified normal thermometer.

Immediately after the zero point determined the one junction was pushed into the anal opening (2.5 cm. deep) and the resulting deflection was recorded.

The amount of deflection due to the insertion of the junction into the alimentary canal of the earthworms gives at once the temperature difference between the two characters under consideration. A single observation was accomplished within 5 minutes, as the temperature of water can not be kept absolutely constant beyond this period.

For the purpose of comparision, the same procedure was applied to dead as well as to decapitated specimens.

MATERIALS.

The materials used were *Pheretima megascolidioides* (Goto &

HATAI), and were collected chiefly in the vicinity of the city of
Imaharu in Shikoku, though these are commonly found in Japan.
Some of the materials were collected in the neighbourhood of Naga-
machi in Sendai.

Though we have abundant earthworms of other forms, for example,
Ph. comminissima, *Ph. vittata* etc., which are easily collected near
Sendai, *Ph. megascolidioides* was chosen on account of its larger size,
slow movement and best of all the fact that they do not exihibit the
usual jumping or twisting movement when the junction is inserted
into the alimentary tract but remain quiet. The materials collected
from Shikoku and Sendai were kept in a big wooden tub into which
earth was filled. The specimens used were apparently adult forms
possessing well developed clitellum; the worms weighed from 8 to 9
gramms and measured from 8 to 10 mm. in diameter in the clitellar
region. I also used the other common species of earthworm, *Ph.
comminissima*, but I could not find any noticeable difference in the
response from that of *Ph. megascolidioides*, and unless otherwise
stated the following statements apply solely to the observations made
on the latter species.

<div align="center">RESULTS OBTAINED</div>

The observations were made on specimens of a nearly identical
body weight of about 6 gramms. There were vigorous and fully
matured, as can be judged from the possession of a clitellum, and
the materials were collected in Spring and Autumn in the vicinity of
the city Imaharu, Southern Japan.

In all cases the worms were kept in ordinary tap water and unless
otherwise stated the temperature difference means the differences
between the body and tap water.

The observations were continued from Sept. 2 up to Nov. 6 and
I have also recorded the condition of the weather and the room
temperature as well as the body weight of each specimen. While
the experiment was going on, if the worm exhibited a sign of weak-
ness, it was immediately discarded; that is, vigorous specimens alone
were used.

The total of 194 observations made on 57 normal specimens are
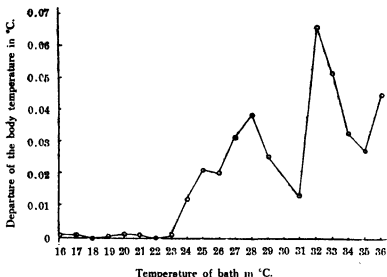given in Table 1.

TABLE 1. Showing the amount of departure of the body temperature from that of the medium in normal specimens.

Galvanometer readings in mm.	0	.5	1,0	1.5	2,0	2.5	3,0	3.5	4,0	4,5	5,0	5,5	6,0	6,5	7,0	7,5	8,0	9,0	9,5	10,0	10,5	11,0	11,5	12,0	16,0	20,0	25,0	30,0	32,0	33,0
Departure of body temperature in °C.	0	.002	.005	.0C7	.010	.012	.015	.017	.020	.022	.025	.027	.030	.032	.035	.037	.040	.045	.050	.060	.075	.1C0	.105	.125	.150				.160	.1R0
16		5	2																											
17		8	2																											
18		8	2(1)	(1)																										
19		6																												
20		13	6(1)																											
21		21	4																											
22		12	(6)																											
23		18	5																											
24							1																							
25								1																						
26															1															
27								1				1				1														
28				1					1			3		1		3														
29				3					2			1		3		1		1												
30										1 1)				9		3		1												
31				1				1	1	3		1		8		3		1	1			1								
32		8		3						2		1		9		2		1	2			1							2	
33		3					1		3			1		8		1		1	1	6		1							1	1
34		3							1			1		6		1		1												1
35		4			1		1					2		1		1		2												
36			2			1	1		2			2																		

Temperature of medium in °C.

Parenthesis represents the frequency of departure of the body temperature lower than that of the medium.

Fig. 1. Based on Table 1. The ordinate indicates the amount of departure of the body temperature, and the abscissa the temperature of the bath.

As will be seen in Table 1. and Fig. 1. the earthworms adjust their body temperature to that of the medium quickly and regularly when the temperature of the later ranges from 16° to 23°C. while beyond 23°C. or between 24–36°C. the adjustment of the body temperature to the medium becomes slower and more irregular as it rises. Indeed, at the lower range of temperature adjustment takes place within 3 min. while at a higher temperature range it requires more than 4 min. Furthermore in the majority of cases a permanent difference between the temperature of the body and of the medium, though higher, is clearly shown. It thus appears that the earthworm is able to adjust its body temperature to that of the surrounding medium quickly and closely within the range between 16°–23°C. and the amount of departure which exists does not much exceed 0.005°C., but is usually much less than that value.

The small data so far obtained indicate that even below 16° to 12°C. the worms respond in nearly the same way as those at 16°–23°C.; that is with a quick and regular adjustment of one to the other.

I tested the power of resistance of the earthworms in tapwater

at temperatures ranging from 15.5°C. A specimen of 13 gramms body weight survived as long as 22 days, while another specimen having 6 gramms body weight survived 6 days at about 6°C.

There is an indication that the worm can survive longer at a lower temperature, as another used showed a survival of 12 days at from 7° to 13°C.

In general the thermal responses of the earthworms narcotized with 0.5% Chloreton at 18.2°C. or even dead specimens (not narcotized) require a longer period before the temperature reading of both the external medium and the inside of the worm become identical; i. e., over 4 minutes, as a rule, instead of within 3 minutes as in the normal healthy worms.

Lower and Upper Fatal Temperature. — At –0.8°C. the earthworms can move very slowly, but at –2.3°C. the entire body of the worm freezes within 63 sec. (taken by stop-watch). If such frozen worms are suddenly exposed to the room temperature (14.5°C.) they move round vigorously within 3 minutes. At –2.3°C. the worms can survive so as to carry on three observations without difficulty. At –11.3°C. the worm freezes within 12 sec. and in no instances was revival noted after once being frozen at this temperature, even if transfered immediately to the room temperature. The above observations were taken in 13 specimens which body weight of 5 gramms on the average.

At 32°C. the earthworms are usually torpified within 47 minutes (the thermal response at this temperature is given in Table 1.), and at 37°C. are torpified within 1.5 minutes. And when these are removed to the room temperature (14°C.) revival takes place within 2 minutes. At 61°C. the earthworms are torpified within 17 seconds and no recovery takes place after return to the room temperature. The above observations at higher temperatures were taken from 34 specimens having body weight of 6 gramms on the average.

It was found that sudden removal of the anterior segments produces little or no difference in body temperature compared with the temperature indicated immediately before its decapitation, though in a few instances an elevation of body temperature amounting to some 0.002°C. was noted, but it soon returned to the former level. Thus the decapitated specimens also behave similarly to the intact specimen in so far as the ability of adjusting the body temperature to that of

the surroundings is concerned. This seems to indicate that the temperature regulation of body is not controled by the higher nervous center or brain.

From these various reactions, it seems safe to say that within the temperature range of from 6° to 23°C. the earthworm can perform a normal life process.

VERNON (1897) found that the CO_2 output of cold-blooded animals shows but slight increase on warming newts or earthworms (*L. terrestris*) from 10° to 22.5°C. The observation of VERNON seems to agree with my own above result.

And also my results agree closely with HATAI's observations (1922) referring to the effect of heat on the rhythmic contractions of earthworms; i. e., he noted that the normal from until the temperature reached 23°C., but over this point the form of contraction alters suddenly.

Beyond 24°C. the temperature difference between the body and medium enhances and at 32° and 33°C. it shows the maximum difference. Over 33°C. the difference rather diminishes than increases.

When the temperature rises, the mechanical response of earthworms is altered remarkably, owing probably to the heightened muscle tone. Somewhat increased heat production due to such abnormal muscle contraction is either dissipated instantly or it may remain temporarily in the muscle, giving an increased heat value in the body over that in the medium. In other words, the ability in regulating the body temperature to that of the surroundings steadly decreases as the temperature rises beyond 24°C.

CONCLUSION.

(1) The body temperature of the earthworms is promptly adjusted to that of the surrounding medium within the temperature range of from 6° to 23°C., while beyond 24°C. its ability of regulation steadily and progressively decreases as the temperature rises.

(2) The regulation of the body temperature of earthworms is little influenced by the nervous center or brain.

(3) It seems that in the earthworm the ability of keeping its proper body temperature against unsuitable environmental temperature in water is little developed.   Dec. 1929

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Distribution of the Intermuscular Nerve Cells in the Earthworm.

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(With 8 Text-figures)

INTRODUCTION.

In this work I desired to determine the number and distribution of the so-called "intermuscular nerve cells" in the earthworm and to furnish data for interpreting the physiological significance of these cells from their numerical relations.

The presence of the intermuscular nerve cells of the earthworm has been noted by several investigators and mentioned incidentally in several papers on the central and peripheral nervous systems of this animal. Recently Dawson ('20) described in detail the shape, size and situation of these cells in *Allolobophora caliginosa* and *A. foetida* but the number as well as the distribution of these cells was not stated.

According to Dawson, the intermuscular nerve cells are the scattered nerve cells, probably vestiges of the primitive nerve net of lower invertebrates.

It is generally accepted that the diffuse peripheral nervous system of the lower invertebrates was transformed into the centralized deep-lying system of the higher invertebrates and vertebrates (Parker, '19, pp. 204-205) and, therefore, the distributional arrangement of these cells may give us some hints on the functional explanation of these cells.

MATERIALS AND METHOD.

The materials used for the present work were three species of *Pheretima : Ph. communissima* (Goto and Hatai), *Ph. megascolidioides* (Goto and Hatai) and *Ph. vittata* (Goto and Hatai), and one species of *Allolobophora : A. foetida* (Sav.). These species are found in

abundance in northern Japan. They were collected at Sendai during
July and August, in which period most of these animals are matured.
I have used the matured animals alone and paid attention in my
investigation to the three following points.

    1. The distribution of the intermuscular nerve cells in the four
regions, head, clitellum, middle and tail region of *Ph. communissima*,
in order to gain a general notion as to the distributional arrangement
on the whole body.

    2. The distribution of these cells in the middle region of the
three species of *Pheretima: Ph. communissima, Ph. megascolidioides*
and *Ph. vittata*, in order to determine the similarity or dissimilarity
the manner of distribution in different species of the same genus.

    3. The distribution of these cells in the middle region of *A.
foetida* for the purpose of comparing its arrangement between the
two different genera.

    After being fed with filter paper for several days to discharge
the earth from the digestive canal, the worm was narcotized for a
few minutes in a solution of 0.1% chloretone. The entire specimen
was fixed in acetic-sublimate solution (Saturated sublimate 100 parts
and glacial acetic acid 5 parts) for three hours, and washed in running
water for about 6 hours. The remaining sublimate was removed with
iodin-tincture, and then washed in running water for about 6 hours
again. After the washing, the necessary parts were removed from
the body and, after being embedded in paraffin in the ordinary
method, were cut 10 micra thick in serial sections. It was found
from preliminary tests that the section of 10 μ thickness is convenient
for distinguishing and counting the nerve cells. The sections were
stained first with DELAFIELD's Haematoxylin followed by an aqueous
solution of Eosin.

THE SHAPE OF THE INTERMUSCULAR NERVE CELLS.

    From their external shapes these cells may be grouped into five
types, i. e. spindle shaped bipolar cells (Fig. 1), crescent-shaped bipolar
cells (Fig. 2. A), tripolar cells (Fig. 2. B), long, slender, pyramidal,
or spindle shaped cells (Fig. 2. C) and multipolar cells (Fig. 2. D).
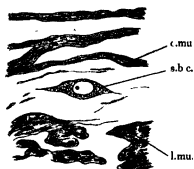Of these five types the first four have already been described by

Fig. 1. Spindle shaped bipolar cell c.mu, circular muscles; l.mu., longitudinal muscles, s.b c. spindle shaped bipolar cell ×700
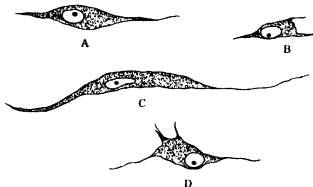

Fig. 2. A. Crescent-shaped bipolar cell. B. Tripolar cell C Long, slender, pyramidal, or spindle shaped cell D. multipolar cell ×700.
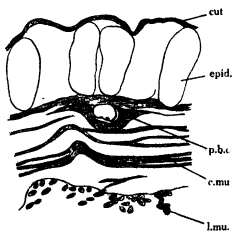

Fig. 3. Particular typed bipolar cell. c.mu., circular muscles; cut., cuticula; epid., epidermis; l.mu., longitudinal muscles; p.b c., particular typed bipolar cell. ×700.

Dawson ('20). The number of bipolar cells is greatly in excess of the number of other forms. In *A. foetida* many spindle shaped bipolar cells different from those just mentioned in the above are found (Fig. 3).

THE SITUATION OF THE INTERMUSCULAR NERVE CELLS.

These cells are present either between the longitudinal and circular muscle layers or between the epidermis and circular muscle layer, and even, sometimes, within the circular muscle layer. In the three species of *Pheretima* these cells are found mainly between the longitudinal and circular muscle layers, and rarely in the two other regions mentioned above. In *A. foetida* the particular spindle shaped bipolar cells are abundantly found within the circular muscle layer near the epidermis and especially between the epidermis and circular muscle layer.

THE DISTRIBUTION OF THE INTERMUSCULAR NERVE CELLS

It is obvious that in order to understand the manner of distribution of these intermuscular nerve cells along the entire body of the earthworm, the preparation of complete serial sections is required, but at the same it is equally obvious that such procedure can not be easily practiced, especially when several worms with considerably elongated bodies are to be compared. As a consequence, I have prepared complete serial sections of one segment each from the four different parts of the body and counted the total number of cells contained in those segments. At the same time I have examined the entire serial sections which were prepared from *Ph. vittata* and also the serial sections made from more than one half of the entire body of *Ph. megascolidioides* and have gained a strong impression that the manner of distribution of these cells along the entire body axis is very similar with an exception of slight tendency of gradual reduction in the frequency of occurrence of these cells antero-posteriorly.

The actual counting of the cells in the segments chosen shows that, in *Ph. communissima* (Table I and Charts 1 & 2), certain individuals have the cells more concentrated both in the head and the tail regions, while other individuals show no such regional concentration but are nearly the same in all four regions examined. On the parts

TABLE I.

No of Sections	Pheretima communissima — no. of individuals								No of Sections	Pheretima communissima — no. of individuals							
	1				2					1				2			
	Situation of segment				Situation of segment					Situation of segment				Situation of segment			
	Head region (III)	Clitellum region (XIV)	Middle region (XXXXV)	Tail region (LXXXVIII)	Head region (III)	Clitellum region (XIV)	Middle region (XXXXV)	Tail region (LXXXVIII)		Head region (III)	Clitellum region (XIV)	Middle region (XXXXV)	Tail region (LXXXVIII)	Head region (III)	Clitellum region (XIV)	Middle region (XXXXV)	Tail region (LXXXVIII)
1	—	—	—	—	1	—	—	—	42	—	—	1	3	1	—	2	—
2	1	—	1	—	1	—	—	1	43	—	—	1	1	—	1	1	—
3	—	—	—	—	—	—	—	1	44	—	—	—	1	—	1	—	—
4	—	—	—	—	—	—	—	—	45	1	—	—	—	—	—	—	—
5	—	—	—	1	—	—	—	—	46	—	—	1	—	—	—	—	—
6	—	—	1	—	—	—	—	1	47	—	—	1	—	—	—	—	—
7	—	—	—	1	—	—	—	—	48	—	—	—	—	—	—	—	—
8	—	—	—	1	—	—	—	—	49	—	—	—	—	—	—	—	—
9	—	—	—	1	—	—	—	—	50	—	—	—	—	—	—	—	—
10	—	—	—	—	—	—	—	—	51	—	—	—	—	—	—	—	—
11	1	—	2	—	—	1	—	—	52	—	—	—	2	—	—	—	—
12	—	—	—	—	1	2	—	—	53	—	—	—	—	—	—	—	—
13	1	—	—	—	—	—	—	—	54	—	—	—	—	—	—	—	—
14	—	—	—	2	2	2	—	—	55	—	—	—	—	—	—	—	1
15	2	—	—	1	1	—	1	—	56	—	—	—	—	—	—	—	—
16	1	—	—	1	1	—	1	—	57	—	—	—	—	—	—	—	—
17	—	—	—	—	1	—	—	—	58	—	—	1	1	—	—	—	—
18	1	—	2	—	1	1	2	—	59	—	—	—	—	—	—	—	—
19	1	—	—	1	1	—	—	—	60	—	—	—	—	—	—	—	—
20	1	—	1	—	1	—	—	—	61	—	—	—	—	1	—	—	—
21	1	—	—	3	1	1	—	—	62	—	—	—	—	—	—	—	—
22	—	—	—	1	1	—	1	—	63	—	—	—	—	1	—	—	2
23	—	—	—	1	1	1	—	—	64	—	—	—	—	1	—	—	1
24	—	1	—	1	1	—	—	—	65	—	—	—	—	—	—	—	—
25	3	—	—	2	2	2	—	—	66	1	—	—	—	—	—	—	—
26	1	—	—	—	1	—	—	—	67	—	2	—	—	—	—	—	2
27	—	1	—	1	1	—	—	—	68	1	—	—	—	—	—	—	1
28	2	—	—	1	1	—	—	—	69	1	—	3	—	—	—	—	—
29	1	—	—	1	1	—	—	—	70	1	—	1	—	—	—	—	—
30	3	—	—	—	1	—	—	2	71	—	—	—	—	—	—	—	—
31	—	—	—	—	—	—	—	2	72	—	—	1	—	—	—	—	—
32	1	—	—	—	1	1	—	1	73	—	—	2	—	—	—	—	—
33	1	—	1	—	1	1	1	—	74	2	1	—	—	—	—	—	3
34	—	—	1	—	—	—	—	1	75	1	1	1	—	1	—	—	—
35	—	—	2	4	1	—	—	—	76	2	—	—	—	1	—	—	1
36	—	—	2	—	—	1	—	—	77	1	1	1	—	1	—	—	1
37	—	—	2	—	1	—	—	—	78	2	—	—	—	2	—	1	—
38	1	—	1	—	1	—	—	—	79	—	—	—	—	—	—	—	—
39	1	—	1	—	1	1	—	—	80	4	—	—	1	—	1	—	—
40	1	—	—	1	2	—	3	—	81	2	—	—	—	1	—	—	1
41	1	—	—	1	2	—	—	—	82	2	—	—	—	1	—	—	1

| No of Sections | Pheretima communissima — no of individuals | | | | | | | |
| | 1 — Situation of segment | | | | 2 — Situation of segment | | | |
	Head region (III)	Clitellum region (XIV)	Middle region (XXXXV)	Tail region (LXXXVIII)	Head region (III)	Clitellum region (XIV)	Middle region (XXXXVIII)	Tail region (LXXXVIII)
83	1	—	1	—	—	—	—	—
84	—	—	—	—	—	—	—	—
85	1	—	—	—	—	—	—	1
86	4	—	1	—	2	—	—	—
87	2	1	—	—	1	—	1	2
88	2	—	—	—	2	—	—	—
89	—	—	—	—	—	—	—	1
90	—	2	—	—	—	—	—	—
91	—	—	—	—	—	—	1	—
92	—	—	—	—	1	—	—	—
93	—	—	—	—	2	—	—	—
94	—	—	—	—	—	—	—	—
95	—	1	—	—	1	—	—	—
96	—	2	—	—	—	—	—	—
97	—	—	—	1	—	—	—	—
98	1	1	—	1	1	—	1	—
99	—	—	—	1	2	—	—	—
100	—	—	—	—	—	—	—	—
101	1	—	—	1	—	—	1	—
102	—	1	—	—	—	—	—	—
103	—	—	—	—	1	—	1	—
104	1	—	—	—	1	—	—	—
105	—	—	1	—	—	—	1	—
106	—	1	1	—	—	—	—	—
107	—	—	1	—	—	—	—	—
108	—	—	1	—	—	—	—	—
109	—	1	—	—	—	—	—	—
110	—	1	—	—	—	—	1	—
111	1	—	—	—	—	—	—	—
112	—	—	1	—	1	—	—	—
113	1	1	1	—	1	—	1	—
114	—	1	—	—	—	—	—	—
115	—	—	1	—	—	—	1	—
116	1	1	3	—	—	—	—	—
117	—	—	—	—	—	—	2	—
118	—	—	1	—	—	—	2	—
119	—	1	1	—	2	—	1	—
120	—	—	1	—	—	—	—	—
121	—	1	—	—	—	—	—	—
122	—	—	—	—	—	—	—	—
123	—	—	—	—	—	—	—	—
124	—	—	—	—	1	—	—	—
125	—	—	—	—	1	—	—	—
126	—	—	—	—	—	—	—	—

| No of Sections | Pheretima communissima — no of individuals | | | | | | | |
| | 1 — Situation of segment | | | | 2 — Situation of segment | | | |
	Head region (III)	Clitellum region (XIV)	Middle region (XXXXV)	Tail region (LXXXVIII)	Head region (III)	Clitellum region (XIV)	Middle region (XXXXVIII)	Tail region (LXXXVIII)
127	—	—	—	—	1	—	—	—
128	—	—	—	—	1	—	—	—
129	—	—	—	—	1	—	—	—
130	—	—	—	—	2	—	—	—
131	—	—	—	—	—	—	—	—
132	1	—	—	—	—	—	—	—
133	—	—	—	—	—	—	—	—
134	—	—	1	—	2	—	—	—
135	—	—	1	—	—	—	—	—
136	—	—	—	—	—	—	—	—
137	—	—	2	—	2	—	—	—
138	—	—	—	—	—	—	—	—
139	—	—	—	—	—	—	—	—
140	—	—	—	—	1	—	—	—
141	—	—	1	—	1	—	—	—
142	—	—	—	—	—	—	—	—
143	—	—	2	—	—	—	—	—
144	—	—	—	—	1	—	—	—
145	—	—	—	—	—	—	—	—
146	—	—	—	—	—	—	—	—
147	—	—	—	—	1	—	—	—
148	—	—	1	—	—	—	—	—
149	—	—	1	—	1	—	—	—
150	—	—	1	—	—	—	—	—
151	—	—	—	—	—	—	—	—
152	—	—	—	—	—	—	—	—
153	—	—	—	—	1	1	—	—
154	—	—	—	—	1	—	—	—
155	—	—	—	—	—	—	—	—
156	—	—	—	—	—	—	—	—
157	—	—	—	—	—	2	—	—
158	—	—	—	—	1	—	—	—
159	—	—	—	—	—	—	—	—
160	—	—	—	—	—	—	—	—
161	—	—	—	—	1	1	—	—
162	—	—	—	—	1	1	—	—
163	—	—	—	—	—	—	—	—
164	—	—	—	—	—	—	—	—
165	—	—	—	—	—	—	—	—
166	—	—	—	—	—	—	—	—
167	—	—	—	—	—	—	—	—
168	—	—	—	—	—	—	—	—
169	—	—	—	—	—	—	—	—
170	—	—	—	—	—	—	—	—

Name of species — *Pheretima communissima*

No. of Sections	Head region (III)	Clitellum region (XIV)	Middle region (XXXV)	Tail region (LXXXVIII)	Head region (III)	Clitellum region (XIV)	Middle region (XXXVIII)	Tail region (LXXXVIII)
171	—				—			
172	—				—			
173	—				—			
174	—				—			
175	—				—			
176	—				—			
177	—				—			
178	—				—			
179	—				—			
180	—				—			
181	—				—			
182	—				—			
183	—				—			
184	—				—			
185	—				—			
186	—				—			
187	—				—			
188	2				—			
189	—				—			
190	—				—			
191	1				—			
192	—				—			
193	—				—			
194	—				—			
195	—				—			
196	—				—			
197	2				—			
198	—				—			
199	1				—			
200	1				—			
201	1				—			
202	1				—			
203	—				—			
204	2				—			
205	—				—			
206	1				—			
207	—				—			
208	2				—			
209	1				—			
210	—				—			
211	1				—			
212	—				—			
213	—				—			
214	—				—			
215	—				—			
216	1				—			
217	—				—			
218	—				—			
219	1				—			
220	1				—			
221	1							
222	2							
223	—							
224	1							
225	—							
226	2							
227	—							
228	—							
229	—							
230	—							
231	—							
232	—							
233	—							
234	—							
235	—							
236	—							
237	—							
238	—							
239	—							
240	—							
241	—							
242	—							
243	—							
244	—							
245	—							
246	—							

near the setae and in the intermuscular regions apart from the nerve ring there are very few. The number of these cells within one segment tends to decrease toward the tail (Table II and Chart 3).

Chart 1.

Tail region (LXXXVIII)

Middle region (XLV)

Clitellum region (XIV)

Head region (III)

No of sections per segment
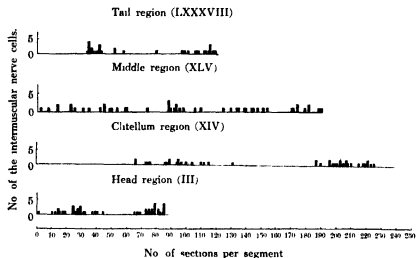
Chart 1   Based on Table I and shows the distribution of the intermuscular nerve cells in the four regions of the body of *Ph. communissima* (1st individual)

Chart 2.

Tail region (XCVIII)

Middle region (XLVIII)

Clitellum region (XIV)

Head region (III)

No. of sections per segment.
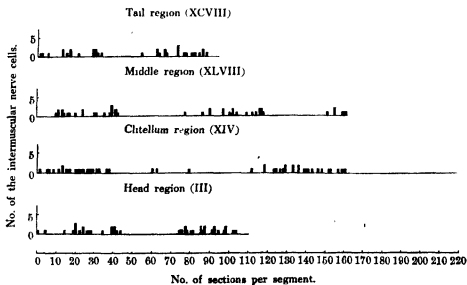
Chart 2. Based on Table I and shows the distribution of the intermuscular nerve cells in the four regions of the body of *Ph. communissima* (2nd individual).

TABLE II.

Ph. communissima.

No. of individuals	Situation of segment			
	Head region	Clitellum region	Middle region	Tail region
	no. of nerve cells per segment	no. of nerve cells per segment	no. of nerve cells per segment	no. of nerve cells per segment
1	49	45	50	36
2	41	50	44	34
Average	45	47.5	47	35

Chart 3.

No. of the intermuscular nerve cells

Regions of the body.

Head region — Clitellum region — Middle region — Tail region
(III) — (XIV) — (XLVII) — (XCIII)
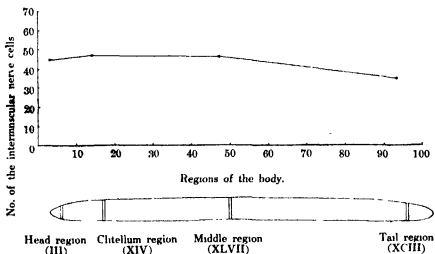
Chart 3. Based on Table II and shows the decrease in the number of the intermuscular nerve cells toward the tail in *Ph. communissima.*

I have counted the total number of cells contained in one segment in the middle region of the body of the three species of *Pheretima* and have also determined the manner of distribution. As will be seen from Tables III & IV and Chart 4 there are no differences in the manner of distribution though there is a difference in the number of cells contained. *Ph. megascolidioides* has the largest number (52.5), *Ph. communissima* next (47) to it and *Ph. vittata* has the least (36).

TABLE III.

No. of sections	Ph. com.		Ph. meg.		Ph. vittata		Allolobophora foetida			
	no. of indiv.		no. of indiv.		no. of indiv.		no. of individuals			
	1	2	1	2	1	2	1		2	
	Situation of seg.	Situation of seg.	Situation of seg.	Situation of seg.	Situation of seg.	Situation of seg.	Situation of seg.		Situation of seg.	
	Middle region (XXXXV)	Middle region (XXXXVIII)	Middle region (LVI)	Middle region (LVI)	Middle region (LII)	Middle region (LII)	Middle region (XXXXVII)		Middle region (XXXXVII)	
							Ordinary typed C.	Particul. typed C.	Ordinary typed C.	Particul. typed C.
1	—	—	1	—	—	—	—	1	—	1
2	1	—	—	1	—	—	—	3	—	3
3	—	—	—	—	—	—	—	4	—	—
4	—	—	—	—	—	—	—	1	—	3
5	—	—	1	—	1	—	—	1	—	2
6	1	—	—	1	—	1	1	2	—	2
7	—	—	—	1	—	1	1	2	1	1
8	—	—	—	1	—	—	1	—	—	1
9	—	—	1	—	—	2	2	1	—	3
10	—	—	—	—	—	—	2	1	—	2
11	2	1	—	—	—	—	1	1	—	2
12	—	2	1	—	—	—	2	1	—	3
13	—	—	—	—	1	1	1	—	—	3
14	—	2	—	—	1	2	—	1	—	2
15	—	1	—	—	—	—	—	—	—	2
16	—	—	—	—	—	—	—	—	—	2
17	—	1	1	—	2	—	—	—	—	1
18	2	1	1	—	—	—	—	1	—	—
19	—	—	—	—	—	—	—	—	—	1
20	1	—	1	—	—	—	—	3	—	—
21	—	1	—	—	—	—	—	1	—	3
22	—	—	—	—	—	—	—	2	—	2
23	—	—	—	—	—	—	—	1	—	1
24	1	—	—	1	—	—	—	3	—	1
25	—	2	1	—	3	—	—	1	—	1
26	—	—	1	—	1	—	—	1	—	1
27	1	—	2	—	1	—	—	2	—	1
28	—	—	1	—	1	—	—	4	—	3
29	—	—	—	1	—	—	—	3	—	1
30	—	—	—	—	—	—	—	1	—	1
31	—	—	1	—	2	—	—	4	—	1
32	—	1	1	—	—	—	—	1	—	3
33	1	—	—	—	—	—	—	3	—	4
34	—	—	—	—	—	—	1	3	—	—
35	2	—	1	—	—	—	1	1	—	3
36	—	1	—	1	—	1	1	5	—	1
37	—	—	—	—	—	—	—	1	—	2
38	1	—	—	1	—	—	—	2	—	—
39	1	1	1	—	—	—	—	3	—	—

No. of sections	Name of species									
	Ph. com.		Ph. meg.		Ph. vittata		Allolobophora foetsda			
	no of indiv.		no. of indiv.		no of indiv.		no of individuals			
	1	2	1	2	1	2	1		2	
	Situation of seg.	Situation of seg.	Situation of seg	Situation of seg	Situation of seg.	Situation of seg	Situation of seg.		Situation of seg	
	Middle region (XXXXV)	Middle region (XXXXVIII)	Middle region (LVI)	Middle region (LVII)	Middle region (LLVI)	Middle region (LLII)	Middle region (XXXXVII)		Middle region (XXXXVII)	
							Ordinary typed C.	Particul. typed C.	Ordinary typed C.	Particul typed C.
40	—	3	—	1	—	1	—	1	—	2
41	—	—	1	—	—	—	—	—	1	1
42	1	2	—	—	—	1	—	3	—	2
43	—	1	—	—	—	—	1	—	1	2
44	—	—	—	—	—	—	1	—	—	—
45	—	—	—	—	—	—	1	—	—	1
46	1	—	—	—	—	—	—	1	—	6
47	1	—	—	—	—	—	—	)	—	1
48	—	—	—	—	—	1	—	1	1	—
49	—	—	—	—	—	—	—	3	—	4
50	—	—	—	—	—	—	—	—	—	2
51	—	—	—	—	—	—	1	2	2	—
52	—	—	—	—	—	—	—	2	—	3
53	—	—	—	—	—	—	—	2	—	1
54	—	—	1	—	—	—	—	3	—	J
55	—	—	—	—	—	—	1	—	—	4
56	—	—	1	—	—	—	—	1	—	1
57	—	—	1	—	—	1	2	4	—	1
58	1	—	1	1	—	—	3	—	1	—
59	—	—	—	—	—	—	1	—	—	—
60	—	—	—	—	—	1	1	—	—	—
61	—	—	—	—	—	1	1	1	—	7
62	—	—	—	—	1	1	1	4	—	8
63	—	—	—	—	—	4	3	6	—	5
64	—	—	—	—	—	1	—	2	—	3
65	—	—	1	—	—	1	—	3	—	4
66	—	—	1	—	—	1	—	5	—	4
67	—	—	—	1	—	—	1	1	—	4
68	—	—	1	—	—	1	—	3	—	4
69	3	—	—	—	—	1	—	1	—	1
70	1	—	—	1	—	1	—	2	—	1
71	—	—	—	—	—	—	—	3	—	4
72	1	—	—	1	—	—	—	3	—	2
73	2	—	1	2	—	—	—	1	—	3
74	—	—	1	1	1	—	—	1	—	1
75	1	—	—	1	—	—	—	—	—	1
76	—	—	1	—	2	—	—	—	—	1
77	1	—	1	—	—	—	—	—	—	2
78	—	1	1	—	1	—	—	—	—	2
79	—	—	—	—	—	—	—	—	—	2
80	—	—	—	—	2	—	—	—	1	2

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No. of sections	Ph. com 1 Middle region (XXXXV)	Ph. com 2 Middle region (XXXXV)	Ph. meg. 1 Middle region (LVI)	Ph. meg. 2 Middle region (LVI)	Ph. vittata 1 Middle region (LII)	Ph. vittata 2 Middle region (LII)	Allolobophora foetida 1 Ordinary typed C	Allolobophora foetida 1 Particul typed C.	Allolobophora foetida 2 Ordinary typed C.	Allolobophora foetida 2 Particul typed C
81	—	...	1	—	—	—	—	—	—	4
82	—	...	1	—	—	—	—	—	—	3
83	1	...	—	—	1	—	—	—	—	2
84	—	...	—	—	1	—	—	3	—	2
85	—	...	—	—	—	—	—	3	—	3
86	1	...	—	1	—	—	—	3	—	3
87	—	1	—	—	—	—	1	1	—	1
88	—	...	—	1	2	—	3	1	—	3
89	—	...	1	—	—	—	4	—	—	4
90	—	—	1	—	—	—	3	3	—	1
91	—	1	—	1	1	—	1	1	—	2
92	—	...	1	—	1	—	—	1	—	1
93	—	...	—	1	2	—	2	2	—	2
94	—	...	—	2	1	—	2	1	—	2
95	—	...	—	—	1	—	1	4	—	5
96	—	...	1	—	1	—	—	2	—	3
97	—	...	1	—	—	—	—	1	—	—
98	1	1	—	1	1	—	—	—	—	2
99	—	—	—	1	1	—	—	3	—	2
100	—	...	1	—	1	—	—	3	—	1
101	—	1	—	1	—	—	—	1	—	1
102	1	—	—	—	1	—	—	2	—	—
103	—	1	—	—	—	—	—	—	—	—
104	—	...	—	—	—	—	1	—	—	4
105	1	1	—	1	1	—	—	1	—	2
106	1	—	—	1	1	—	—	1	—	3
107	—	...	—	—	—	—	—	1	—	1
108	—	...	—	1	—	—	—	—	—	—
109	1	...	—	—	—	—	—	1	—	4
110	1	1	—	—	—	—	—	2	—	—
111	—	...	1	1	—	—	—	1	—	1
112	—	...	—	—	1	—	—	—	—	4
113	1	1	—	—	—	—	—	1	—	1
114	1	—	1	—	1	—	—	2	—	3
115	—	1	—	—	—	—	1	—	—	3
116	1	—	—	—	—	1	—	1	—	2
117	—	2	2	—	—	—	1	1	—	1
118	—	2	3	—	—	—	—	—	—	2
119	1	1	1	—	—	—	—	—	—	8
120	—	—	—	—	—	—	—	—	1	—
121	1	—	1	—	—	1	—	—	—	2

No. of sections	Ph. com 1 — Situation of seg, Middle region (XXXXV)	Ph. com 2 — Situation of seg, Middle region (XXXXVIII)	Ph. meg 1 — Situation of seg, Middle region (LVI)	Ph. meg 2 — Situation of seg, Middle region (LVI)	Ph. vittata 1 — Situation of seg, Middle region (LII)	Ph. vittata 2 — Situation of seg, Middle region (LII)	A. lolobophora foetida 1 — Ordinary typed C	A. lolobophora foetida 1 — Particul typed C	A. lolobophora foetida 2 — Ordinary typed C	A. lolobophora foetida 2 — Particul typed C
122	.	—	2	--	—	—			1	3
123	-	—	-		—	1			1	2
124	—	—	-		-				1	3
125	-	—	1		-	1			--	2
126	-	-							--	3
127	-	-	1		—	2			--	—
128	—	-				2			—	—
129	—	-			—				1	1
130	—	-								
131	.	—	2		—				1	3
132	—	—	1	-	—	1			1	---
133	.				-					
134	1	—							1	2
135	1	—			2				1	1
136	-	-	2	-					—	1
137	2	-			1				—	2
138	—								1	2
139	—								—	1
140	—		2		—				—	—
141	1	-	2	1					--	5
142	—	—								
143	2	—	--							
144	-	—								
145	-	-	--							
146	.	—	2							
147	-	—	1							
148	1	—	—							
149	1	-	—							
150	1	-	—							
151		-	—							
152		-	—							
153		1	—							
154		-	1							
155			1							
156			1							
157		2	—							
158			1							
159		—	---							
160		—	—							
161		1	—							
162		1	2							

No of sections	Ph. com no. of indiv. 1 Situation of seg Middle region (XXXXXV)	Ph. com no. of indiv. 2 Situation of seg Middle region (XXXXVIII)	Ph. meg no of indiv. 1 Situation of seg M iddle region (LVI)	Ph. meg no of indiv. 2 Situation of seg Middle region (LVI)	Ph. vittata no of indiv. 1 Situation of seg Middle region (LII)	Ph. vittata no of indiv. 2 Situation of seg Middle region (LII)	Allolobophora foetida no. of individuals 1 Situation of seg Middle region (XXXXVII) Ordinary typed C.	Allolobophora foetida 1 Particul typed C.	Allolobophora foetida 2 Situation of seg Middle region (XXXXVII) Ordinary typed C.	Allolobophora foetida 2 Particul typed C.
163										
164		1		2						
165				—						
166				—						
167				1						
168				—						
169				—						
170				—						
171				—						
172				1						
173										
174										
175										
176				—						
177										
178				1						
179				2						
180				—						
181				1						
182				1						
183										
184				—						

TABLE IV.

No of individuals	Ph. megacolidioides no of nerve cells per segment in middle region	Ph communissima no. of nerve cells per segment in middle region	Ph. vittata no. of nerve cells per segment in middle region
1	58	50	40
2	47	44	32
Average	52.5	47	36

Chart 4.

Middle region (XLVII) of *A. foetida* (1st individual)

Middle region (XLVII) of *A. foetida* (2nd individual)

Middle region (LII) of *Ph. vittata* (1st individual)

Middle region (LII) of *Ph. vittata* (2nd individual)

Middle region (LVI) of *Ph. megascolidoides* (1st individual)

Middle region (LVI) of *Ph. megascolidoides* (2nd individual)

Middle region (XLV) of *Ph. communissima* (1st individual)

Middle region (XLVIII) of *Ph. communissima* (2nd individual)

No. of the intermuscular nerve cells.

0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160 170 180 190

No. of sections per segment.

■ Ordinary typed cells.   ■ Particular typed cells.

Chart 4. Based on Table III and shows the distributional arrangement of the intermuscular nerve cells in the middle region of the body of the three species of *Pheretima* (*Ph. communissima*, *Ph. megascolidoides* and *Ph. vittata*) and one species of *Allolobophora* (*A. foetida*).

No of the intermuscular nerve cells.

- ▦ Ph. vittata
- ⠿ Ph. communissima
- ▩ Ph. megascolidioides
- ■ A. foetida (ordinary typed cells)
- ▨ A. foetida (particular typed cells).
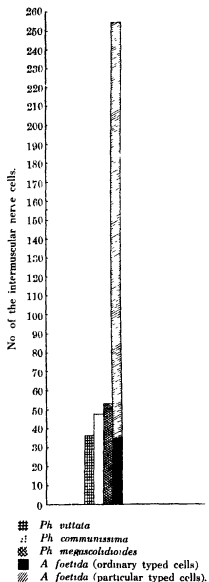
Chart 5   Based on Table V and shows the comparison of the number of the intermuscular nerve cells in the middle region of the body among the three species of Pheretima (Ph. communissima, Ph. megascolidioides and Ph. vittata) and one species of Allolobophora (A. foetida).

A. foetida shows that the number of ordinary typed cells (34.5) is very similar to that found in the three species of Pheretima, but the particular typed cells are much more numerous (219.5) (Tables III & V and Charts 4 & 5).

From the fact that the intermuscular nerve cells show no different grade of concentration in the different parts of the body, and since they are so few in number, we may be safe to conclude that these cells probably play no important functional role.

If it were granted that these cells are vestiges of the primitive nerve net of lower invertebrates as DAWSON claims, the nervous system of A. foetida is more primitive in its evolution than that of the species belonging to the genus Pheretima.

TABLE V.

No. of individuals	Name of species				
	Ph. megas-colidoides	Ph. com-munissima	Ph. vittata	A. foetida	
	no. of nerve cells per segment in middle region	no. of nerve cells per segment in middle region	no. of nerve cells per segment in middle region	no. nerve cells per segment in middle region	
				Ordinary typed cells	Particular typed cells
1	58	50	40	50	105
2	47	44	32	19	274
Average	52.5	47	36	34.5	219.5

SUMMARY

1. In the three species of *Pheretima*, *Ph. communissima*, *Ph. megascolidoides* and *Ph. vittata*, intermuscular nerve cells are found as they are in *Allolobophora caliginosa* and *A. foetida*.

2. In the three species of *Pheretima* these cells are found mainly between the longitudinal and circular muscle layers and, rarely, between the epidermis and circular muscle layer or within the circular muscle layer. In *Allolobophora foetida* the particular spindle shaped bipolar cells are numerous within the circular muscle layer near the epidermis, and also immediately under the epidermis.

3. The distribution of these cells in *Ph. communissima* shows no remarkable difference in concentration on the whole body. But the number of these cells tends to decrease antero-posteriorly along the body.

4. Among the three species of *Pheretima*, *Ph. megascolidoides* has the largest number of these cells, *Ph. communissima* next to it, and *Ph. vittata* has the least.

5. *A. foetida*, though it possesses nearly the same number of the ordinary typed cells, has many more of the particular typed cells than of the ordinary, in contrast to the three species of *Pheretima*.

6. The distributional arrangement as well as the insignificant number of these cells seem to suggest that these cells in the earthworm are vestiges of the primitive nerve net of lower invertebrates as DAWSON claims, and probably play no functional role.

I should like here to express my hearty thanks to Prof. S. HATAI for his kind guidance and valuable suggestions during the course of this work.

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Effect of Inorganic Salts on Photic Orientation in *Allolobophora foetida* (SAV.)

5. Sodium Salts — Na2SO4, NaNO3, and NaCl

By

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(With 6 Text-figures).

ABSTRACT. The experiments were carried on by submerging the worms in a solution of the single or mixed sodium salts.

1. In the ventral nerve cord, NaNO3 and NaCl caused a weakening of positively orienting functioning, while Na2SO4 caused a strengthening at first and then a weakening

2. In the brain, NaNO3 caused a weakening, NaCl a strengthening, and Na2SO4 a strengthening at first and then a weakening, of negatively orienting functioning.

3. In Na2SO4 + NaNO3, the change in positive orientation tended mainly to follow that of the worms placed in NaNO3, while the change in negative orientation showed a tendency between those occurring in Na2SO4 and NaNO3 separately.

4. In Na2SO4 + NaCl, the change in positive orientation tended mainly to follow that in NaCl, but the change in negative orientation appeared to follow neither that in Na2SO4 nor in NaCl.

5. In NaNO3 + NaCl, the change in both orientations tended mainly to follow that in NaNO3.

6. In all the single and mixed solutions, backward crawling appeared to be caused mainly by a relative weakening of forward crawling functioning in the ventral nerve cord. Winding movement might be more or less pronounced

So far as we had carried our experiments on the effect of inorganic salts on the photic orientation in *Allolobophora foetida*, among salts used[1-4], we found one, NaCl, which caused a weakening of positively

1) MgCl2, CaCl2, NaCl, and KCl. Sci. Rep. Tohoku Imp Univ., 4th Ser, Vol 3, No. 2, Pp. 151-177. Methods of the experiments and of treating data are given in this paper.

2) MgSO4, FeSO4, Na2SO4, and K2SO4. Ibid., Vol. 3, No. 3, Fasc. 1, Pp. 223-248.

3) Mg(NO3)2, Ca(NO3)2, NaNO3, and KNO3. Ibid, Vol 3, No 3, Fasc 2, Pp 379-403.

4) NaI, KI, NaBr, and KBr. Ibid., Vol. 3, No 4, Fasc. 1, Pp 647-663

orienting functioning in the ventral nerve cord and a strengthening
of negatively orienting functioning in the brain, therefore a strength-
ening of the degree of negative orientation in the worms as a whole.
In the present experiments we are going to test again the effect of
NaCl[1] and, in connection with this, of other sodium salts, Na$_2$SO$_4$[1]
and NaNO$_3$[1].

The control experiments were carried on on the 17th and the 31st
of August, 1928, separately, each in a temperature of 25°C. The
data given in this paper were the averages of the results from these
two sets of experiments.

I EFFECT OF SINGLE SALTS.

The experiments were carried on in a temperature of 25°C., August
17–23, 1928 In the experiments, 1/2 the normal solutions of NaNO$_3$
and NaCl, and 1/4 the normal solution of Na$_2$SO$_4$ were used separately.

A Movements of Unoperated Worms.

50 worms were tested individually in a definite solution for each
duration of submergence, viz. 30, 60, 90, and 120 seconds.

In Na$_2$SO$_4$, the worms showed neither convulsion nor ejection of
coelomic fluid, and they were still vivid even after an elapse of 800
seconds.

In NaNO$_4$, the worms showed convulsion as soon as they were
submerged, but ejection of coelomic fluid would begin generally after
an elapse of 13 or more seconds. When the duration of submergence
was prolonged above 250 seconds most of the worms could not crawl
out.

In NaCl, at a duration of about 25 seconds, the worms showed
convulsion which was in most cases followed directly by ejection of
coelomic fluid. Sometimes a second set of convulsion and ejection
was observed. They were very sluggish at a duration above 450
seconds.

The differences in number of seconds found between the previous
and present experiments in NaNO$_4$ and NaCl might presumably
depend upon the difference of concentration, as well as of temperature
under which the experiments were made.

TABLE 1.

Angles occupied by the unoperated worms.

	Duration of submergence in seconds	5 cm angles in degrees			10 cm angles in degrees		
		Positive	Average	Negative	Positive	Average	Negative
Na₂SO₄	0	80.43	102.02	111.59	78.37	111.00	122.63
	30	82.64	99.06	106.42	82.88	106.74	113.86
	60	77.44	88.96	101.62	78.54	100.62	112.08
	90	81.34	95.68	104.24	81.62	105.70	114.08
	120	71.74	82.82	101.08	76.32	93.02	106.70
NaNO₃	0	80.43	102.02	111.59	78.37	111.00	122.63
	30	77.62	100.30	112.68	78.46	107.46	119.00
	60	71.40	86.06	104.66	71.42	88.60	107.18
	90	75.68	87.08	101.40	74.36	92.18	107.82
	120	81.00	88.94	97.94	75.38	91.28	105.90
NaCl	0	80.43	102.02	111.59	78.37	111.00	122.63
	30	75.86	99.78	113.92	73.92	111.02	127.10
	60	76.50	100.12	113.62	71.10	108.22	127.12
	90	77.30	108.00	120.70	75.32	120.52	135.20
	120	79.84	113.98	124.04	76.16	122.16	136.00

TABLE 2.

Frequency distribution of the unoperated worms.

	Duration of submergence in seconds	5 cm. angles			10 cm. angles		
		0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
Na₂SO₄	0	10	6	34	11	4	35
	30	10	13	27	8	14	28
	60	17	14	19	14	14	22
	90	12	18	20	13	14	23
	120	25	10	15	20	11	19
NaNO₃	0	10	6	34	11	4	35
	30	16	9	25	14	7	29
	60	25	10	15	23	10	17
	90	20	10	20	21	7	22
	120	18	14	18	20	8	22
NaCl	0	10	6	34	11	4	35
	30	11	3	36	12	4	34
	60	10	6	34	13	4	33
	90	8	6	36	10	5	35
	120	8	5	37	9	4	37

Orientation.

From Tables 1 and 2 it may be inferred that, in Na₂SO₄ and NaNO₃, the degree of negative orientation of the worms tended to weaken with the increase of the number of seconds of submergence, while in NaCl it tended to strengthen.

Crawling.

Though in all the solutions (Table 3), the number of backward crawling and winding individuals might be increased after the submergence, in the present experiments [these phenomena were shown only very feebly.

TABLE 3.

Frequency of crawling of the unoperated worms.

	Duration of submergence in seconds	5 cm angles					10 cm angles			
		Forward			Backward					
		Directly	After posterior elongation		Directly	After anterior elongation	Forward	Backward	Returning	Winding
Na₂SO₄	0	50	0	0	0	0	50	0	0	0
	30	50	0	0	0	0	50	0	0	0
	60	50	0	0	0	0	50	0	0	1
	90	50	0	0	0	0	50	0	0	0
	120	50	0	0	0	0	50	0	0	0
NaNO₃	0	50	0	0	0	0	50	0	0	0
	30	50	0	0	0	0	50	0	0	0
	60	46	0	0	4	0	46	4	0	0
	90	50	0	0	0	0	50	0	0	0
	120	50	0	0	0	0	50	0	0	0
NaCl	0	50	0	0	0	0	50	0	0	0
	30	50	0	0	0	0	50	0	0	0
	60	49	0	0	1	0	49	1	0	0
	90	50	0	0	0	0	50	0	0	0
	120	50	0	0	0	0	50	0	0	0

B. Movements of Operated Worms.

25 worms were tested individually for each duration of submergence.

Orientation.

From Tables 4 and 5 it may be noted that Na_2SO_4 tended to cause in the worms a strengthening at first and then a weakening of the degree of positive orientation, while $NaNO_3$ and $NaCl$ tended to cause a successive weakening.

TABLE 4.

Angles occupied by the operated worms.

	Duration of submergence in seconds	5 cm. angles in degrees			10 cm. angles in degrees		
		Positive	Average	Negative	Positive	Average	Negative
Na_2SO_4	0	59.18	65.00	95.82	47.02	55.16	98.14
	30	52.88	56.92	94.04	43.52	50.04	96.52
	60	59.20	65.08	95.88	46.64	53.72	97.08
	90	59.92	62.88	92.96	48.68	52.96	94.28
	120	62.52	67.52	95.00	52.08	60.04	97.96
$NaNO_3$	0	59.18	65.00	95.82	47.02	55.16	98.14
	30	62.48	68.84	96.80	53.88	53.32	99.44
	60	69.56	79.·4	99.88	64.52	76.96	102.64
	90	70.22	80.68	100.96	68.92	82.08	106.16
	120	72.96	85.28	102.92	66.96	84.36	107.40
$NaCl$	0	59.18	65.00	95.82	47.02	55.16	98.14
	30	56.16	61.32	95.16	48.24	54.84	96.60
	60	56.48	62.92	96.44	47.16	55.20	99.04
	90	58.88	67.56	98.68	52.52	61.20	98.68
	120	60.56	69.36	98.80	54.04	64.04	100.36

TABLE 5.

Frequency distribution of the operated worms.

	Duration of submergence in seconds	5 cm. angles			10 cm. angles		
		0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
Na_2SO_4	0	17	4	4	19	2	4
	30	20	2	3	22	0	3
	60	18	3	3	19	2	4
	90	17	5	3	19	4	3
	120	17	4	4	17	4	4

Duration of submergence in seconds	5 cm angles			10 cm angles		
	0°-80°	81°-99°	100°-180°	0°-80°	81°-99°	100°-180°
NaNO₃ 0	17	4	4	1	2	4
30	18	6	4	16	2	7
60	12	5	8	12	4	9
90	11	6	8	12	4	9
120	9	8	8	11	4	10
NaCl 0	17	4	4	19	2	4
30	14	4	7	16	2	7
60	13	4	8	15	4	6
90	12	5	8	14	3	8
120	13	3	9	16	1	8

Crawling.

According to Table 6, in all the cases the number of backward crawling individuals was increased after the submergence. The number of winding individuals might be also increased.

TABLE 6.

Frequency of crawling of the operated worms.

	Duration of submergence in seconds	5 cm angles				10 cm angles			
		Forward		Backward		Forward	Backward	Returning	Winding
		Directly	After posterior elongation	Directly	After anterior elongation				
Na₂SO₄	0	9	2	13	1	11	14	0	0
	30	11	0	14	0	10	15	0	1
	60	11	0	11	3	11	14	0	0
	90	8	0	17	0	8	17	0	0
	120	10	1	12	2	11	14	0	1
NaNO₃	0	9	2	13	1	11	14	0	0
	30	0	0	25	0	0	25	0	1
	60	5	0	20	0	5	20	0	1
	90	5	0	20	0	5	20	0	0
	120	6	1	17	1	7	18	0	0
NaCl	0	9	2	13	1	11	14	0	0
	30	2	0	21	2	2	23	0	2
	60	2	1	22	0	3	22	0	1
	90	3	1	20	1	4	21	0	0
	120	6	0	19	0	6	19	0	1

C. Changes of Negativity in the Brain.

From Table 7, Figs. 1-3 were plotted in order to see easily the states of the changes. In these figures the tracings of the 5 cm. angles are denoted by the broken lines, and those of the 10 cm. angles by the full lines.

TABLE 7.

Calculation of N.

	Duration of submergence in seconds	5 cm angles in degrees			10 cm angles in degrees		
		P	A	N	P	A	N
Na₂SO₄	0	65.00	102 02	127.02	55.16	111.00	145.84
	30	56.92	99.06	132.14	50.04	106.74	146.70
	60	65.08	88.96	113.88	53.72	100.62	136.90
	90	62.88	95 58	122 70	52.96	105.70	142.74
	120	67.52	82.82	105.30	60.04	93.02	122 98
NaNO₄	0	65.00	102.02	127.02	55.16	111.00	145.84
	30	68 84	100.30	121.46	63.32	107.46	134.14
	60	79.24	86.06	96.82	76.96	88.60	101 64
	90	80.68	87.08	96.40	82.08	92.18	100.10
	120	85.28	88.94	93.66	84.36	91.28	96.92
NaCl	0	65.00	102.02	127.02	55.16	111.00	145.84
	30	61.32	99.78	128.46	54.84	111.02	146.18
	60	62.92	100.12	127.20	56.20	108.22	142.02
	90	67.56	108.00	130.44	61.20	120.52	149.32
	120	69.36	113.88	131.52	64.40	122.16	147.76

Na₂SO₄ (Fig. 1) tended to cause in the brain a strengthening at first and then a weakening of the degree of negative orientation, though this tendency was not shown in the present experiments so clearly as it was in the previous.

NaNO₄ (Fig. 2) appeared in the present experiments to cause in the brain only a successive weakening of the degree of negative orientation, without causing a strengthening at an early part of the submergence which was shown in the previous experiments. From the lack of data at a duration shorter than 30 seconds, however, we are unable to judge whether the result of either the previous or the present experiments is correct or not.

NaCl (Fig. 3) tended to cause in the brain a strengthening of
the degree of negative orientation in coincidence with the results of
the previous experiments.

Fig. 1   Na₂SO₄.
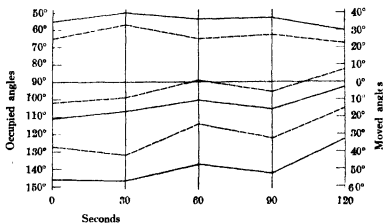
Fig. 2   NaNO₃.
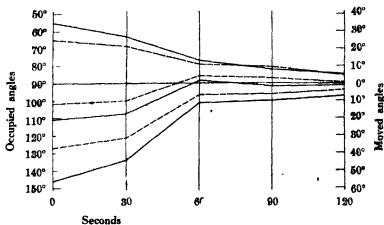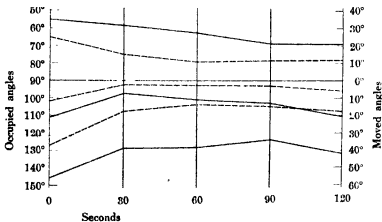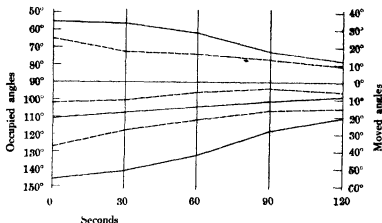
Fig. 3. NaCl.
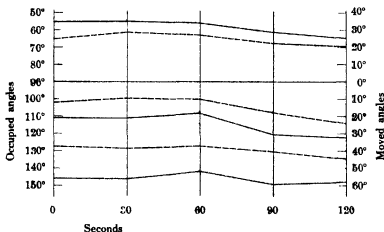
D. Changes in Crawling.

From Tables 3 and 6 we may infer that, in all the solutions used, the strengthening of backward crawling functioning in the ventral nerve cord is caused mainly by a relative weakening of forward crawling functioning in the ventral nerve cord itself. This statement coincides with those given in our preceding papers.

II EFFECT OF MIXED SALTS.

The experiments were carried on in a temperature of 25°C., August 23-28, 1928. For an experiment, a given amount of 1/2 the normal solutions of NaNO₃ and NaCl and of 1/4 the normal solution of Na₂SO₄ was mixed with the same amount of one of the other solutions.

A. Movements of Unoperated Worms.

50 worms were tested individually for each duration of submergence.

In Na₂SO₄ + NaNO₃, the worms showed convulsion at a submergence of about 6 seconds, and ejection of coelomic fluid about 250 seconds. When the worms were submerged above 750 seconds they could hardly crawl out.

TABLE 8.

Angles occupied by the unoperated worms.

	Duration of submergence in seconds	5 cm angles in degrees			10 cm. angles in degrees		
		Positive	Average	Negative	Positive	Average	Negative
Na₂SO₄ + NaNO₃	0	80.43	102.02	111.59	78.37	111.00	122.63
	30	81 06	110 00	118.94	70.18	114.08	124.90
	60	79.40	100.40	111.00	80.46	109.74	110.28
	90	79.94	98.42	108.48	80.02	104.88	114 26
	120	75.70	89.08	103.38	77.30	97.20	109 90
Na₂SO₄ + NaCl	0	80 43	102.02	111.59	78.37	111.00	122.63
	30	78.41	92.57	104.16	75.75	97.16	111.41
	60	79.96	93.08	103.12	77.82	101.12	113.30
	90	78.06	93.06	105.00	78.44	103.00	114.56
	120	81.83	95 76	105.93	80.02	110.56	120.54
NaNO₃ + NaCl	0	80.43	102.02	111.59	78.37	111.00	122 63
	30	80.06	100.28	110.22	78.62	107.32	118.70
	60	79.16	95.70	106.54	76.22	104.04	117.82
	90	78.24	93.40	105.16	78.16	100.98	112.82
	120	79.28	95.68	106.40	78.82	98.30	109.48

In Na₂SO₄+NaCl, at about 200 seconds of submergence the worms showed convulsion which was directly followed by ejection of coelomic fluid. A second ejection of coelomic fluid was often observed. When the time was prolonged above 1100 seconds the worms became very sluggish.

In NaNO₃+NaCl, the worms showed convulsion soon after the submergence and ejection of coelomic fluid at about 13 seconds. When they were submerged in the solution above 350 seconds they were not able to crawl out.

Orientation.

From Tables 8 and 9 we may infer as follows:

In Na₂SO₄+NaNO₃, the worms tended to show a weakening of the degree of negative orientation. This tendency of change might be taken as an expression between those shown by the worms placed in Na₂SO₄ and NaNO₃ separately.

In NaNO₃+NaCl, the worms showed also a weakening of the degree of negative orientation. This tendency of change thus showed a nearer resemblance to that in NaNO₄.

TABLE 9.

Frequency distribution of the unoperated worms.

	Duration of submergence in seconds	5 cm angles			10 cm angles		
		0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
Na₂SO₄ + NaNO₃	0	10	6	34	11	4	35
	30	8	4	38	8	5	37
	60	15	11	24	12	9	29
	90	16	11	23	13	8	29
	120	18	19	13	19	12	19
Na₂SO₄ + NaCl	0	10	6	34	11	4	35
	30	13	12	25	17	9	24
	60	11	13	26	14	9	27
	90	11	11	28	12	8	30
	120	10	10	30	12	5	33
NaNO₃ + NaCl	0	10	6	34	11	4	35
	30	10	9	31	10	8	32
	60	12	10	28	11	10	29
	90	12	14	24	11	16	23
	120	11	19	20	10	21	19

In Na₂SO₄ + NaCl, the worms showed a weakening at first and then a strengthening of the degree of negative orientation. This tendency appears to follow neither that in Na₂SO₄ nor in NaCl

Crawling.

Though in all the solutions, the number of backward crawling and winding individuals might be increased after the submergence, in the present experiments (Table 10) these phenomena were shown only feebly as they were in the cases of the single salts (Table 3).

B. Movements of Operated Worms.

25 worms were tested individually for each duration of submergence.

Orientation.

According to Tables 11 and 12, in all the solutions the worms tended to decrease the degree of positive orientation.

In Na₂SO₄ + NaNO₃, and NaNO₃ + NaCl, the tendency of change

TABLE 10.

Frequency of crawling of the unoperated worms.

| | Duration of submergence in seconds | 5 cm angles | | | | 10 cm angles | | Returning | Winding |
| | | Forward | | Backward | | | | | |
		Directly	After posterior elongation	Directly	After anterior elongation	Forward	Backward		
Na₂SO₄ + NaNO₃	0	50	0	0	0	50	0	0	0
	30	50	0	0	0	50	0	0	0
	60	49	0	1	0	49	1	0	0
	90	48	0	2	0	48	2	0	1
	120	50	0	0	0	50	0	0	0
Na₂SO₄ + NaCl	0	50	0	0	0	50	0	0	0
	30	50	0	0	0	50	0	0	1
	60	50	0	0	0	50	0	0	0
	90	50	0	0	0	50	0	0	0
	120	50	0	0	0	49	1	0	0
NaNO₃ + NaCl	0	50	0	0	0	50	0	0	0
	30	50	0	0	0	50	0	0	0
	60	50	0	0	0	50	0	0	0
	90	50	0	0	0	50	0	0	0
	120	50	0	0	0	50	0	0	0

TABLE 11.

Angles occupied by the operated worms.

| | Duration of submergence in seconds | 5 cm angles in degrees | | | 10 cm angles in degrees | | |
		Positive	Average	Negative	Positive	Average	Negative
Na₂SO₄ + NaNO₃	0	59.18	65.00	95.82	47.02	55.16	98.14
	30	61.36	66.52	95.16	54.04	61.40	97.36
	60	62.88	69.20	96.32	55.36	62.84	97.48
	90	66.76	71.24	94.48	55.68	61.92	96.24
	120	69.28	73.36	94.08	57.64	64.96	97.32
Na₂SO₄ + NaCl	0	59.18	65.00	95.82	47.02	55.16	98.14
	20	65.64	75.08	99.44	50.40	58.68	98.28
	60	70.72	79.48	98.76	54.20	62.84	98.64
	90	70.88	78.32	97.44	60.44	69.20	98.76
	120	71.60	78.28	96.68	61.80	68.72	97.12
NaNO₃ + NaCl	0	59.18	65.00	95.82	47.02	55.16	98.14
	30	64.20	72.68	98.48	49.76	56.00	96.24
	60	67.00	74.08	97.08	54.08	61.48	97.40
	90	67.68	76.84	99.16	60.28	72.48	102.20
	120	69.64	80.76	101.12	68.04	77.80	99.76

TABLE 12.

Frequency distribution of the operated worms.

	Duration of submergence in seconds	5 cm. angles			10 cm. angles		
		0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
Na₂SO₄ + NaNO₃	0	17	4	4	19	2	4
	30	16	5	4	18	4	3
	60	16	5	4	17	4	4
	90	16	6	3	18	4	5
	120	16	5	4	17	4	4
Na₂SO₄ + NaCl	0	17	4	4	19	2	4
	30	14	6	5	17	3	5
	60	14	5	6	16	4	5
	90	14	6	5	15	4	6
	120	12	9	4	15	5	5
NaNO₃ + NaCl	0	17	4	4	19	2	4
	30	16	4	5	18	2	5
	60	15	5	5	16	3	6
	90	15	3	7	14	3	8
	120	13	4	8	13	4	8

TABLE 13.

Frequency of crawling of the operated worms.

	Duration of submergence in seconds	5 cm angles				10 cm angles			
		Forward		Backward					
		Directly	After posterior elongation	Directly	After anterior elongation	Forward	Backward	Returning	Winding
Na₂SO₄ + NaNO₃	0	9	2	13	1	11	14	0	0
	30	4	0	21	1	4	21	0	1
	60	2	0	23	0	2	23	0	1
	90	1	0	22	2	1	24	0	1
	120	3	0	22	0	5	20	0	0
Na₂SO₄ + NaCl	0	9	2	13	1	11	14	0	0
	30	1	1	22	1	2	23	0	0
	60	3	0	22	0	3	22	0	0
	90	3	0	22	0	3	22	0	0
	120	8	0	17	0	8	17	0	0
NaNO₃ + NaCl	0	9	2	13	1	11	14	0	0
	30	4	1	20	0	5	20	0	2
	60	6	0	19	0	6	19	0	1
	90	3	0	22	0	3	22	0	0
	120	2	0	21	2	2	23	0	0

appeared to be influenced by NaNO₃ more than by Na₂SO₄ or NaCl respectively.

In Na₂SO₄ + NaCl, the tendency might be influenced by NaCl more than by Na₂SO₄.

Crawling.

In all the solutions (Table 13), the number of backward crawling worms increased after the submergence. The number of winding individuals might be also increased.

C. Changes of Negativity in the Brain.

From Table 14, Figs. 4–6 were plotted. In these figures too the tracings of the 5 cm. angles were denoted by the broken lines, and those of the 10 cm. angles by the full lines.

TABLE 14.

Calculation of N.

	Duration of submergence in seconds	5 cm angles in degrees			10 cm angles in degrees		
		P	A	N	P	A	N
Na₂SO₄ + NaNO₃	0	65.00	102.02	127.02	55.16	111.00	145.84
	30	66.52	110.00	133.48	61.40	114.08	142.68
	60	69.20	100.40	121.20	62.84	109.74	136.90
	90	71.24	98.42	117.18	61.92	104.88	132.96
	120	73.36	89.08	105.72	64.96	97.20	122.24
Na₂SO₄ + NaCl	0	65.00	102.02	127.02	55.16	111.00	145.84
	30	75.08	92.57	107.49	58.68	97.16	128.48
	60	79.48	93.08	103.60	62.84	101.12	128.28
	90	78.32	93.06	104.74	69.20	103.00	123.80
	120	78.28	95.76	107.48	68.72	110.56	131.84
NaNO₃ + NaCl	0	65.00	102.02	127.02	55.16	111.00	145.84
	30	72.68	100 28	117.60	58.00	107.32	141.32
	60	74.08	95.70	111.62	61.48	104.04	132.56
	90	76.84	93.40	106.56	72.48	100.98	118.50
	120	80.76	95.68	104.92	77.80	98.30	110.50

Na₂SO₄ + NaNO₃ (Fig. 4) caused in the brain a weakening of the degree of negative orientation. This tendency of change might be

taken as an expression between those of the worms placed in Na_2SO_4 and $NaNO_3$ separately.

$Na_2SO_4 + NaCl$ (Fig. 5) caused in the brain a weakening at first and then a strengthening of the degree of negative orientation. This tendency appeared to resemble neither that in Na_2SO_4 nor $NaCl$.

$NaNO_3 + NaCl$ (Fig. 6) caused in the brain a weakening of the degree of negative orientation. This tendency appeared to be influenced much stronger by $NaNO_3$ than by $NaCl$.

Fig 4 $Na_2SO_4 + NaNO_3$.
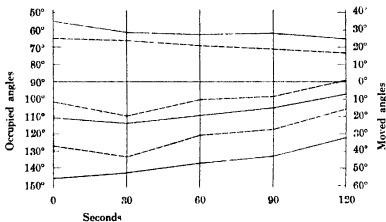
Fig 5 $Na_2SO_4 + NaCl$

Fig. 6   NaNO₃+NaCl.

D.   Changes in Crawling.

From Tables 10 and 13 we may infer that, in all the mixed solutions used, the strengthening of backward crawling functioning is caused mainly by a relative weakening of forward crawling functioning in the ventral nerve cord.

III.   SUMMARY

Na_2SO_4 caused a strengthening at first and then a weakening of both positively orienting functioning in the ventral nerve cord and negatively orienting functioning in the brain. Convulsion and ejection of coelomic fluid were not observed.

$NaNO_3$ caused a weakening of both positively and negatively orienting functionings. Convulsion began soon after the submergence, and ejection of coelomic fluid after an elapse of about 13 seconds.

NaCl caused a weakening of positively orienting functioning, and a strengthening of negatively orienting functioning. Convulsion occurred at about 25 seconds and in most cases it was followed directly by ejection of coelomic fluid.

$Na_2SO_4 + NaNO_3$ caused a weakening of both positively and negatively orienting functionings. Convulsion occurred at about 6 seconds, and ejection of coelomic fluid at about 250 seconds.

$Na_2SO_4 + NaCl$ caused a weakening of positively orienting functioning, and a weakening at first and then a strengthening of negatively orienting functioning. Convulsion began at about 200 seconds, being followed by ejection of coelomic fluid.

$NaNO_3 + NaCl$ caused a weakening of both positively and negatively orienting functionings. Convulsion began soon after the submergence, and ejection of coelomic fluid at about 13 seconds.

In $Na_2SO_4 + NaNO_3$, the change in positive orientation tended mainly to follow that of the worms placed in $NaNO_3$, while the change in negative orientation showed a tendency between those occurring in Na_2SO_4 and $NaNO_3$ separately.

In $Na_2SO_4 + NaCl$, the change in positive orientation tended mainly to follow that in $NaCl$, but the change in negative orientation appeared to resemble neither that in Na_2SO_4 nor in $NaCl$.

In $NaNO_3 + NaCl$, the change in both orientations tended mainly to follow that in $NaNO_3$.

In all the single and mixed solutions used, a strengthening of backward crawling was caused mainly by a relative weakening of forward crawling functioning in the ventral nerve cord.

In all the cases winding movement might be more or less pronounced.

May 24, 1930.

On *Drawida hattamimizu*, Sp. Nov.

By

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(With 7 Text-figures)

The presence of this gigantic semi-aquatic species of Oligochaeta belonging to the genus *Drawida* is said to have been known for years to the people living in the villages near Kahoku Lake in the prefecture of Ishikawa which is facing, and opens by a narrow inlet to, the Japan Sea. This worm is extensively used by the local fishermen as a live bait for eel fishing and is called by them Hattamimizu, distinguishing it from other earthworms.

A few years ago, a school master of Hatta village, Mr. Tetsujiro Mori, sent this worm to Professor To Ichimura, of the Fouth Government High School at Kanazawa, who in turn sent it to Professor Seitaro Goto at Tokyo Imperial University for identification. First of all I wish to thank Prof. Goto for intrusting to me the study of this interesting worm, and I also wish to extend my hearty appreciation to the other two gentlemen mentioned above who not only made this worm known outside of their locality, but, during my visit later to this lake for further collection of materials, gave me first hand assistance in accomplishing the object of my expedition. Taking this opportunity, I wish also to thank the Saitô Hôon Kai (Saito Gratitude Foundation) for the generous financial aid which was given to the collection of materials and facilitated my carrying on this piece of work to a succesful end. I am deeply indebted, too, to my assistant, Mr. Takeye Araya, who accompanied me on this expedition and by whose endurance and painstaking care only I was enabled to collect many specimens in a perfect condition.

The generic characters of *Drawida* are well defined now and even the minute structures of several species have been well studied already by Beddard, Benham, Bourne, Stephenson and others. Consequently it appears to me rather superfluous to describe in too much

detail the structures of the present species, but I have done so to some extent in as much as the present Japanese species shows structures of interest which are unique and since this is the first species of genus *Drawida* found at a definitely known locality and studied from numerous fresh specimens. *Drawida japonica*, MICH. is, of course, the first described species but its exact locality is not known, though it is considered to be identical with the species found in India, Bahama, China.

The present species are certainly to be classed as water dwellers rather than earth dwellers and indeed are found among the rice roots, banks of ditches or ponds where water freely penetrates, although these worms are able to survive for days during the harvest season, at which period water is removed form the rice fields. At this period we can find hundreds of finger-sized holes along the banks of rice fields, deep inside of which the worms are living This habit of living is dreaded by the farmers since a slight incline of the rice field empties the water through these holes. Despite this injurious behavior, this worm is welcomed by the fishermen as they are the best bait for eels. In the villages some distance from the bay, where eel fishing is not practiced, the farmers are making a concerted effort to prevent the intrusion or introduction of the worm, by for instance inhibiting an exchange or getting young rice plants even they were obliged to obtain from far distant villages during failure of their rice nursery bed.

The movement of the worm is very slugish, though the anterior head region is very actively moved.

The susceptibility of this worm is astonishing, and indeed the worm cut into many small pieces, each less than one inch long, may survive for months. Although I have not actually determined the maximum period of survival, yet there is every indication that most of the pieces may, under favourable condition, survive for a considerably longer period.

In many cases I have noted that even where a longitudinal cut is made along the dorsal line and most of the organs are mutilated, the blood vessels emptied, some portion of the organs semi-decomposed and the entire worm is kept under tap water, yet the body wall of the worm responds to slight mechanical stimulation for as long as

twelve days. Another curious fact noted was that the blood within the heart remains red colored with equal intensity for over a week surrounded by tap water unless injured.

The worms survive better in hydrant water, even at near zero temperature for days than in wet earth.

All these points show that the Hattamimizu with its large size and the many virtues mentioned above would be first class material for various physiological investigations.

As to the breeding seasons and breeding habits nothing is known yet. Like the limicolae the worms coil together when many are placed together, but they differ from them as *Drawida* coil their tails together, protruding their heads while the reverse the phenomenon is shown by true limicolae such as *Tubifex*, *Branchiura* etc.

I EXTERNAL CHARACTERS AND HABITATS

The length of the body of this worm is more difficult to measure than most other terricolae on account of a considerable degree of extensibility and retractibility. When seen in their natural habitat while slowly moving or crawling, the longest so far found elongated itself to as long as three feet yet the posterior portion of its body was not fully extended. If the worm is held with the fingers by its tail and hangs freely downward it often elongates five or six times its normal contracted length. I have given below averaged measurement taken from six well developed worms which were killed with gradually concentrated alcohol and preserved in 85% alcohol.

The length given for this worm is by no means unusual and thus ranks it with most other known gigantic species of this genus. For comparison I shall give measurements of several gigantic worms of known *Drawida* in comparison with this Japanese species.

	Body			Color	Number of gizzards	Clitellum	Locality
	Length	Width	Segm.				
D grandis	520	12	266–480	Without Pigment	5,	XVII–XX	X–XIII	India
D. nilamburensis	750	7	566	Slightly Pigmented	5–6,	XXVI–XXXIII	. . . .	India
D. robusta var ophidioides.	310	7	200	Bluish to Olivegreen	4,	XIII–XVI	. ..	India

D. naduvatamensis	500	5	400	Without Pigment	3,	XV–XVII	.... India
D. hattamimizu	246	9.5	317	Bluish-Black	6–9,	XIII–XVIII	¼IX–½XVI Japan.

The above shows that the Japanese species ranks as the largest known species of *Drawida* in the number of segments as well as in the diameter of body.

Color. The color of the fresh worm appears almost black all over the body but when preserved, for instance in 10% formalin, the upper two-thirds of it is deep bluish black while the remaining ventral one-third is much lighter. If preserved in alcohol much of the pigment fades away and presents a very much lighter, grayish color.

In some, sexually fully matured, the clitellum appears. It exhibits a light pink color which almost completely surrounds the clitellar segments. The color of the younger worm usually appears brick red owing to the lack of the pigment though both the anterior and posterior portions of the body are slightly darker. We however often find apparently adult worms which exhibit a light brick red coloration all over the body instead of bluish black, excepting a slight dark color in both the anterior and posterior regions. This color of bluish black is easily soluble in strongly acidulated alcohol similar to that of *Allolobophora* but differing in the coloration of the *Pheretima* which pigment is insoluble in this reagent.

The prostomium is a prolobish and large round body in preserved specimens.

Papillae. In the ventral surface along the head region, especially in the neighborhood of the sexual openings, one pair of prominent papillae in each segment is present. Though these papillae are easily seen in sexually matured individuals, even in somewhat immatured specimens they can be seen after preservation. In all specimens I have examined so far the arrangement of the papillae is not strictly symmetrical but one or more from either side are usually and irregularly missing. The arrangement of the papillae on the specimens of best provided is as follows:

From VI to IX, one pair in each segment along the inside of the inner setal line:

From XI to XIII, one pair in
each segment along the inside of the
inner setal line. One pair some times
found in X, but usually lacking.

The above arrangements are found
in worms provided with most numer-
ous papillae and indeed we meet
often with specimens having them
only in XI, XII and XIII. Often
however the papillae are entirely
absent externally in the worms which
appear in every respect sexually fully
matured but, after dessection, show
yellowish spots internally where the
papillae should normally be present
externally.

The position of the papillae, if
present, occupies invariably a place
slightly in and behind the inner setal
position. The shape of the papilla
is circular and elevated slightly from
the body surface. The diameter
varies from almost one mm. to one
and a half mm. The tip of the
papilla is usually pointed, though in
some specimens it is flattened.

Each papilla is connected internally
with a patch of glandular structure
which exhibits a deep yellow colora-
tion. This patch of gland is usually
oblong shaped and is found even
when no papillae are discernible ex-
ternally. I have shown its position
in Fig. 4. from which one will notice
that these patches are symmetrically
located not only along the inner setal
lines but, in addition, we often find

Sp.th.op.

Clitellum

Fig 1. Showing the ventral
views of *Drawida hattamimizu*.
Note the presence and arrange-
ments of a large number of papilla.

similar shaped patches located along the outer region of the outer
line or between the inner and outer setal lines.

The sexual markings, such as the presence of papillae, modified
body wall exhibiting swollen patches, grooves etc., are stated to be
weakly developed in the genus *Drawida*. The present Japanese
species of *Drawida* is therefore unique in having so numerous papilla
of prominence and, if constantly present in every individual, this
character alone would enable us to distinguish it from other exotic
species, but unfortunately it is not constantly present in many of
specimens so far examined

Clitellum. The clitellum is comparatively poorly developed in this
species of *Drawida* and is nothing more than a slight modification
of the epidermal layer. The clitellum is hardly visible in a fresh
condition and only becomes evident, if present, after preservation
with either alcohol or formalin. The color of the clitellum is light
pink but it is easily distinguishable from the other segments. The
clitellum is composed of from seven to eight distinct segments and
occupies the region from IX to XV or XVI, thus differing from all
other known exotic species in which it is stated in the majority of
cases to occupy X–XIII, and never more than 5 consecutive segments.
Often the first clitellar segment begins in the posterior half of IX
and ends in the anterior half of the segment XVI.

The setae are present in all the segments except the first and
second, and are similar in size and shape.

Considering the worm as a whole, all the segments after the 8th
gradually narrowed posteriory when compared with the few segments
of the immediate front.

Setae. The shape of the setae is usually sigmoid, Fig. 2., measur-
ing about 50 μ long and 2 μ wide.
It seems to be similar all over the
body and no specially modified
setae in the region of the genera-
tive openings are present. Setal
lines are slightly elevated from the
general surface along the ventral
surface and two setae usually pro-
ject from each row, though often

Fig. 2. Showing the shape of setae.

three setae project.

The relative positions and shape of
the setae are shown in Fig. 3.

Secondary Annulation of Segments.
If the specimens are preserved nicely
the secondary annulation of a permanent
nature is not visible, but when the
worms are abnormally shortened by
strong contraction or the reproductive
organs became fully matured the se-
condary annulation with a deep groove
becomes visible on the ventral surface.

Fig 3 Showing the relative
positions of the setae

It seems to me, so far as my observations go, that permanent second-
ary annulation normally does not exist in this Japanese species.

Dorsal Pores. Similarly to practically all other known species of
Drawida, the present species also totally lacks the dorsal pores.

Nephridiopores. Curiously enough the openings of nephridia
are not visible even with moderately high magnification of binoculars
although I examined carefully, both fresh as well as with best pre-
served, numerous specimens and although it is stated in regard to
other exotic specimens that these are readily seen in the majority of
cases in the neighbourhood of the outermost setal prominence. Micro-
scopical examination of a cross section alone reveals the position of
the nephridial openings. The size of the opening when seen from
sections measures only $1,5 \mu$ to 2μ in diameter.

Sex openings.

Spermathecal Openings. The spermatheca opens along the inter-
segmental line between VII/VIII on the tip of the prominent
papillae, slightly interior to the outermost setal line. The openings
are very distinct even in a somewhat immature specimen. In well
developed worms these papillae on whose tops the openings are found
are conical in shape and the tips bend caudal-ward, slightly covering
the anterior end of VIII. In several specimens the papillae were
absent and the openings were seen to be a mere slit along the inter-
segmental line.

Male Openings. Vas deferens also opens on the top of conspicu-
ously elevated papillae. The openings are located slightly interior

to the outermost setal line between X/XI. The papillae on whose top the openings are found bend often caudal-ward, thus covering a smaller portion of the anterior XI segment. This opening is also very distinct in most specimens.

In some specimens, no papillae such as described in the above were noted, owing probably to the immaturity of the sex glands in those particular specimens.

Female Openings. Close along the intersegmental line, between XI and XII but slightly caudal to them and slightly outside of the innermost setal line the female openings are situated.

The openings are rather inconspicuous and unless the specimens are well preserved its recognition is difficult. Its opening is a mere slit on the body surface and I never found any dermal alteration such as papillae associated with this opening. The openings may be noticed if the worms are hardened with ZENKER's fluid in a well stretched condition, by pinning the walls.

The position of the ovidual pores or female openings in my specimens is puzzling since it is one of the chief generic characters that the openings are located along the intersegmental line XI/XII, though in several species of minute size or of less well preserved specimens several investigators failed to determine the exact position of the openings but assumed that they were at the normal position.

Among the genus belonging to the Moniligastraedae familly, having the ovidual openings within the segment and not along the intersegmental line, is *Eupolygaster* in which the openings are found in XIII segment. However, the Japanese species differs very greatly in other respects from *Eupolygaster* and its inclusion to *Drawida* can not be doubted though in this character of the female openings, it differs from the generally accepted generic characters of *Drawida*, but instead agrees with the former.

The chief external characteristics of *Drawida hattamimizu* are as follows:

The clitellum occupies usually seven segments, IX–XV.

The spermathecal openings are between VII/VIII.

The male openings are between X/XI.

The female openings are in XII, slightly caudal to the intersegmental line XI/XII.

The genital papillae are one pair in each segment in VII, VIII, IX, XI, XII and XIII. In some, the papillae are totally missing.

The size, as well as the form, of the setae is uniform all over the body. These are missing in the first and the last segments.

The average body length of some of the preserved specimens gave 246 mm. with 317 segments. The largest diameter was 9.5 mm.

Nephridiopores are invisible though the position of the ending of the duct is easily located in a dissected specimen in close contact with the outer setal rows.

II. INTERNAL CHARACTERS
(See Fig. 4).

Septum.

The regular septum begins between segments V and VI. Anterior to V there are no typical septa but the surface of the pharynx as well as of the buccal wall is attached by strongly developed fibrous bundles, the other end of which attach to the body wall or some to the septum between V and VI.

These bundles are irregularly attached to the walls concerned but metameric arrangement is loosely shown, that is, these bundles are somewhat grouped together in the corresponding segments.

The septums between VII/VIII, and VIII/IX are very thick and are interwoven with highly glistening fibrous bundles while that at XI/XII is somewhat thinner. Between XI and XXVI the septums exhibit varied thickness, but posterior to XXVI they are very thin.

After the 6th segment the septums do not strictly occupy the intersegmental lines but gradually move caudal-ward and the septum in IX and X occupy almost the middle of the segment X.

After the Xth segment the septums gradually move caudal-ward and all the septums posterior to XII/XIII occupy practically the inter-segmental lines.

The septums between XI/XII and XII/XIII are very thin and soft but from XVI/XVII on till XXII/XIV the septum are considerably thicker with glistening appearance similar to the anterior septums at V/VI-VIII/IX. Why the septums are so thick at these segments is not clear. BEDDARD noted post-gizzard thickening of the septum in *Drawida viridis.*
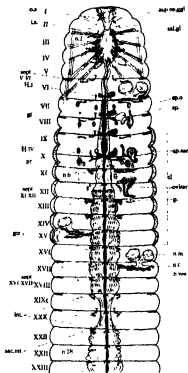
Fig. 4. Showing the arrangement of various internal organs and displacement of some of the septa The setal rows are indicated by dotted lines Three entire nephridiums are shown which for the first, the nephrostomes and ducts which lead to the exterior are alone indicated The part of the ducts drawn by a solid line is exposed in the body cavity while the portion indicated by dotted lines is imbedded within the body wall A thicker septum is indicated by the attachment of narrow rectangles to the regular septum

o s, outer setal line, i.s, inner setal line, sept., septum, l l. first heart, H IV, fourth or last heart, gl, glandular patch, pr, prostate, giz, gizzards, int, intestine, sac, int, saccular intestine, sup oe ggl, supraoesophageal ganglion, sal gl, salivary glands, sp o, spermathecal opening; sp. sac, sperm sac, cl., clitellum, p, membranous pouch, n m, nephredial membrane, n t, nephredial tube, n. ves., nephredial vesicle, n. l. n. R n 18 first, eighth, and eighteenth nephrediums, respectively

No septum is missing as BOURNE ('94) stated in the numerous species of his own discovery. In many adult specimens I have noted a blind sac formation at a pharyngeal part close to the alimentary tract, that is, a conical sac is projected from the rear surface of the septum usually between XII/XIII or often from XIII/XIV additionally. Its dimension as well as position suggests that this sac was formed by the enormous elongation of the posterior end of the egg sac which pushes the septums under consideration caudal-ward thus secondarily forming the permanent sacs which receive the posterior end of the egg sac. I have actually found in several specimens that the egg-sac was held in this sac, but in the majority of cases the egg-sac is not found in this membranous thin sac as the dissection or anaesthetics probably produce severe contraction of the egg-sac thus relieving or drawing out the sacs. It is stated that in several species of *Drawida*,

the egg-sac is enormousely elongated and pushes the septum of several posterior segments, thus forming a sac from the several consecutive septa concerned.

Alimentary Tract.

The mouth opens directly to the buccal cavity which is spacious and its wall is highly extensible. In preserved specimens it presents itself as a narrow tube and occupies the first two segments. The pharyngeal wall is connected to the body wall by numerous strong coarse fibrous bundles, as is the case with most Oligochaetes. The pharyhx occupies the next two segments III and IV, and is followed by a long narrow oesophagus which occupies from the Vth down to the anterior half of the XIIth segments. It is a narrow thin tube and its minute structure shows no especial peculiarity to this species. The oesophagus gradually enlarges as it proceeds caudal-ward and becomes continuous to the first gizzard in the XIIIth segment.

Similarly to other Oligochaetes, the pharyngeal wall is well supplied with well developed salivary glands. There are noted about five pairs of masses of this gland attaching to and surrounding the fibrous bundles mentioned above though I could not find their respective ducts under a dissecting microscope.

The color of the gland is deep pink when fresh owing probably to the rich supply to the blood capillaries. In alcohol or formaline it is white.

The alimentary tract of this species is characterized by the possesion of gizzards the number of which varies with individuals from 6 or $6\frac{1}{2}$ to as many as 9 or $9\frac{1}{2}$ though the individuals with six gizzards are most frequently met with. The first complete gizzard is situated at the XIII segment and the remaining gizzards are found one in each segment. The first gizzard is often weakly developed and under the microscope the muscular wall is thinner and muscular areas are even intermingled with non-muscular areas. In XII, the posterior half exhibits a gizzard-like muscular structure in the majority of specimens so far examined, though its anterior half is a genuine oesophageal structure.

When more than six gizzards are present usually the additional gizzards are found in the posterior segments and in no instances I have found that additional gizzards are found in front of the usual

first location, segment XII. The gizzards are separated from each other by narrow non-muscular areas whose structure resembles that of the oesophagus. It is occasionally found that the portion of the oesophagus which occupy the segment XI or the anterior half of XII are swollen, exhibiting a ring shape but differing from real gizzards not only in color but in softness if touched by a dissecting needle. The external wall of the gizzard appears white while a ring formed by the swelling of the oesophagus appears light pinkish due to the absence of the muscular coat.

The last gizzard is followed by the narrow tubular intestine and occupies the two segments which become continuous to the saccular intestine as in other oligochaetes.

Nephredia. Like all other species of this genus the present species posseses so-called mega-nephredia which attain some considerable dimension especially in those located in the anterior segments, with the exception of the first two pairs which are comparatively small or are rudimentary.

The nephredia are present in all segments with the exception of I, II, IX and XI. Although in IX and XI much smaller vestigial nephredia are often present in an immature specimen, all the tubular portions disappear in a medium sized specimen leaving only the membranous structure in the regular normal position, that is, attaching itself to the posterior surface of septum XI.

The size of nephredia increases progressively down to IV in which segment it reaches the maximum. After IV, the nephredia become somewhat smaller though slightly larger than those found in the segments posterior to XI.

The nephrostome is slightly enlarged and its stalk is longer and projected from the anterior surface of the respective septum, that is, the base of the stalk attaches at the front surface of the septum of each corresponding segment. This stalk becomes continuous immediately behind the septum to the much convoluted . tubules of the glandular structure. At this point where the stalk unites with the tubules a relatively dilated tube of fibrous texture, or the diverticulum is also united. This dilated diverticulum runs together with the glandular tubules while the other end of the diverticulum suddenly narrows and opens outside. (See Figs. 5, 6.)
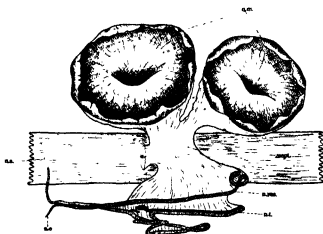
Fig 5. Semi-diagramatical view of the entire nephridium, showing especially the funnel shaped membranous development which short stalks unite together and attach to the septum at a.
sept., septum; n m. nephridial membrane; n s, nephrostome; n o., nephridial opening; n t., nephridial tube of glandular structure; n ves., nephredial vesicle of muscular texture.

The external openings of the nephredia are located along the outer setal line when seen under the microscope but are not detectable externally with even moderately magnified binocular lenses.

Nephredial Openings.

As was stated already, the external openings of the nephredia are indetectable even by means of moderately high powered magnification. Internally however the course of the narrow duct which is connected with the diverticulum into the body wall is very distinct and its opening to the exterior is very distinct under the microscope.

The penetration of this narrow duct into the muscular layer is
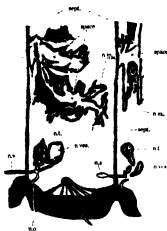
Fig. 6. Diagram of the longitudinal section of one segment showing chiefly the general arrangement of the nephridial system within the segment. A large continuous air space within the nephridial membrane is also shown. The meaning of letterings are the same as in Fig. 3.

interesting. From the anterior II to XI this nephredial duct pene-
trates into the muscular layer along the external setal line and after
running a short distance parallel to the muscular layer bends itself
abruptly and opens externally slightly in front of and also slightly
external to the outer setal line.
(Fig. 7 a.) Under the microscope
the pore opens on a slightly
elevated papillae of conical shape.
The opening is $1.5 \mu - 0.2 \mu$ in
diameter.

The nephredial ducts posterior
to XI penetrate into the muscular
layer along the inner setal line
instead of the outer setal line
and after traversing long distance
along and parallel to longitudinal
muscle layer bend abruptly as
in the former cases and open
at the position slightly in front
and slightly outside of the outer
setal line or, in other words,
externally along the same line
as with the nephredial openings
of the anterior segments. (See
Fig. 7 b.)

Fig 7 Showing the course of the
nephridial vesicular tube which opens to
the exterior in the anterior nine segments
(a) and in the rest of the posterior seg-
ments (b)   al., alimentary canal, gg.,
nervous ganglion, l m, longitudinal mus-
cle layer; c.m., circular muscle layer.
set, setae   For the meaning of other
letterings see the explanation of Fig 4

Another peculiarity of the
nephredial system of this species
is the presence of the three expansive, membranous funnel-shaped,
structures with short common stalk, the base of which attaches to
the posterior surface of the septum.

One of the three funnels envelopes the nephredial tubules by its
edge while the remaining two funnels appear to float freely in the
body fluid expanding like umbrellas. When the worm is dissected
under water along the mid-dorsal line this umbrella-like membrane
immediately floats in the water and, on account of its expansive
dimension together with its snowy white color, attracts the eyes at
once. The histological structure shows that it is chiefly composed of

connective tissue in which granules of fatty-like substance are abun-
dantly seen.

As will be seen from Fig. 6 the cross section of this funnel
structure shows that by the outer and inner walls a space is formed
which evidently serves to expand the umbrella widely or partly.

Minute histological structures of the nephridia are nearly the
same as those already described and illustrated for instances by BOURNE
on *Drawida grandis*. The tubular portion contains two main tubules
which however become single near the nephrostome that is, near
where the diverticulum and glandular tubule unite These two main
tubules already mentioned are in reality composed of the trabeculae
of the minute tubules, as illustrated by BOURNE.

It is interesting that these trabeculae are directly continuous with
the trabeculae contained in expansive membrane in which this system
is very rich. This membrane, then performs some important physio-
logical function though exactly what kind of function it performs can
not be stated. Thus umbrella structure is not always expanded
widely but in some instances it is much shriveled, owing probably to
some adjustment of air space found inside the funnel wall. The
function of this umbrella structure is not clear but the expansiveness
of its top as well as the air space formed within suggests that it
mechanically supports the entire nephredia in proper position by
floating it. The membrane receives very rich supply of the blood
capillaries and is, in turn, again directly continuous with the capillaries
in the nephredial cappillaries. The membrane is chiefly composed to
the mass of cells which are arranged into a group of long continuous
cell strands. Some of these strands contain cells within, which are
filled with lipoidal granules, while in other strands the cells contain
densely packed cytoplasm which stains deeply with almost with any
dye.

Reproductive Organs.

I shall briefly describe the chief characters of each reproductive
organ, though the general arrangement of the reproductive organs is
essentially the same as with other species of the genus.

Spermatheca. One pair of spermatheca is situated in segment
VIII and the saccular portion attaches to the posterior surface of the
septum VII/VIII which is much displaced caudal-ward. The sac is

somewhat circular in shape and its one corner, where a deep groove is formed, is continuous to a very slender tubule. The color of the sac is deep yellow and it can therefore be easily distinguished from other structures. The size of the sac is about 2.5 mm in diameter in an adult specimen.

The tubule is much coiled and then ventrally opens in front of septum VII/VIII along the outer setal line in the intersegmental line between segments VII and VIII. Near the external orifice the tubule runs straight without showing any coil and opens to the papillae which is visible from the outer body surface. The tubule shows a very slight dilation near the terminal and no other modifications are noticeable in connection with this tubular ending.

Testis.

The testis is included within the voluminous sperm sac which is somewhat pear-shaped and appears in front as well as in rear of the septum between IX/X. The round large portion is continuous to the narrowed portion which protrudes caudally through this septum. It therefore appears (though in reality it is one continuous sac) that the sperm sac is composed of two portions, the anterior large round sac and the posterior oblong sac. I may add however that in the worms which were collected during breeding season, the posterior smaller portion was more voluminous and larger than the anterior larger portion. Between these two portions is exhibited a definite groove or constriction where the vas deferens attaches just in front of the septum.

The vas deferens is very slender but strongly coiled especially in front of the septum. The Winding tubules and the greater portion of tubule are located in front of the septum and after much winding penetrate through the basal portion of the septum and appear in the rear of the septum. In this region the tubule again shows some loose winding while progressing caudal-ward but near the intersegmental line between X/XI it returns anteriorly once and then again proceeds posteriorly. This tubule then gradually moves away from the median line towards the outer setal line through the circular muscle layer, where the glandular structure of short oblong shape is located. This structure just stated is the prostate gland which is sparsely covered by a few strands of muscle fibers. The gland appears deep yellow

and covers over the external orifice of the male pore. The vas deferens which entered into the muscle layer, now slightly dilates itself forming an atrium whose opening is found in and under the prostate gland.

The cross section of this gland shows that the vas deferens runs under the glandular structure of the prostate and becomes continuous to a wide and trunkated space, or the atrium.

The anterior end of the vas deferens opens to the anterior large sperm sac in which the testis is attached. The testis is composed of many separated lobes, though these are united and from one common base which is located at the junction of the two portions of the sacs.

Ovaries.

The ovary is quite large in the adult worm, measuring about 5 mm. long and 2 mm. wide, and is freely hung on the posterior surface of the septum X/XI. The shape of the ovary as a whole is somewhat round in its free end. There is no sac formation by the septa or so-called "ovarian sac" surrounding the ovary as is seen with many other species of this genus. Evidently the ripened ova fall off freely and are received into the ovisac.

Ovisac.

The ovisac is very large and conical in shape. Its wider mouth opens into segment XI in front of the septum XI/XII. Posteriorly it narrows and is located in the segment XII. The posterior end of the ovisac is usually slightly bent and is confined in most cases in the segment XII. However I have often noted that the ovisac is much elongated and its posterior end extends as far back as the two consecutive septa XII/XIII, and XIII/XIV by pushing those forming a sac as was illustrated by BOURNE with *Drawida grandis*. In the majority of cases the ovisac contracts itself during anaesthesia or dissection and is evidently drawn out from these secondary sacs just mentioned.

I have even often noted that the ovisac was completely reversed (inside out) suggesting that the ovisac is strongly contractil under external shock.

The size of the ovisac is, in its contracted state, 5 mm. long.

The oviduct is enclosed within the septum XI/XII. It is a slender and much coiled tubule having its outlet slightly caudal of the intersegmental line XI/XII along the inner setal line. The tubules is very

thin-walled, exhibiting a velvety appearance.

Circulatory System.

BOURNE has made very elaborate studies on the circulatory system of *Drawida grandis* and our Japanese species in no particular way differs from the former species and I shall not therefore give any detailed description.

The hearts are found in the four segments from VI to IX inclusive as is the case with all other *Drawida* species. The dorsal vessel is invariably covered thickly by a brownish mass. This mass when seen under the microscope is composed of tall, narrow cells which contain densely packed granules of brownish color and also fat globules. Neither the side branch of the vessel nor delicate vessels are covered by these cells. This cellular covering comes off easily when scratched even gently with forceps or needles.

The chief characteristics of *Drawida hattamimizu* are as follows:

Size, 246 mm. long, 7.4 mm. in diameter.

Segments, 317.

Pigments, deep blue-black though slightly lighter along the ventral side.

Deep pink all over when small, but even in the adult stage pink is found mixed with the blue-black.

Prostomium. — Prolobous.

The clitellum extends from IX to XV or XVI and is pink in color.

Genital papilla are numerousely present in many specimens sexually mature.

One pair is found in each segment from VI to IX and from XI to XIII.

Dorsal pores are absent.

Setae $50\,\mu$ long. Distance between ba-ab wider than dc-ba and dc-cd is slightly over 1/3 circumference. Absent in Segments 1. and 11.

Nephrediopores open in the direction of the outer setal row but are not visible without the aid of high magnification. Nephredia are absent in the first two anterior segments and also in X and XII. In the case of the first eight nephredia the outlet duct first enters into the circular muscle layer at the region in the immediate neighborhood of the outer direction of the outer setal line, then traverse

slightly outwardly before finally opening outside. Posterior to the segment XII the nephredial tube enters into the muscle layer between the outer and inner setal line and traverse beyond the external setal line within the muscle before it opens at the point along the other anterior nephredia.

Another peculiarity of the nephridia is the presence of three expansive double walled membranous structures of funnel shape. A large space is formed between the double walls just mentioned The funnels are attached to the septums concerned by a short flattened common stalk. Two of the funnels are freely exposed but the margin of one encloses the greater portion of the nephridia.

The male pores open in the direction of the outer setal row.

Oviducal pores open in the direction of the inner setal row but are slightly posterior and not in the intersegmental line XI/XII

Spermathecal pores open in the direction of the outer setal row.

Septa, V/VI, VI/VII, VII/VIII and VIII/IX are very thick and again XVI/XVII–XIX/XX are almost equally thickened.

The gizzards are six, occupying the segments XII/XVIII, in majority of cases, but occasionally there are seven or as many as nine. The first gizzard usually beings in segment XII where, in many cases, the posterior half only is thickly coated with muscles. If there are more than six then the additional gizzards are found in the posterior segments and never in the anterior segments.

The spermsac is found both in front and in rear of the septum IX/X. The portion which appears in front is larger round but narrows suddenly and appears in the rear as an elongated sac.

The prostate is oblong and deep yellow when fresh. It adheres directly to the body wall as a sessil glandular patch.

Ovaries are large but not enclosed within the so-called "ovarian chamber".

The copulatory pouch is straight, though slightly dilated from the tube.

The spermathecae is somewhat triangular in shape.

A supraintestinal blood vessel is absent.

Habitat. So far this species has been found exclusively in Hatta and immediately neighboring villages in Kanasawa city along Kahoku lake.

It seems worth while to recapitulate the chief peculiarities presented by the Japanese species contrasted with other species in order to determine the extent of variability of characters within the genus.

The position of the female openings is generally considered as the important generic character distinguishing it from *Eupolygaster*. In all the species so far described if the specimens were matured and the openings were distinctly recognizable, it invariably occurred in XI/XII. There are numerous species recorded in the literature which were studied from immature specimens in which cases the exact position of the female openings were undetermined but were always assumed to be situated in the intersegmental line and not in the segment.

Among the family of Moniligastaedae, the genus *Eupolygaster* possesses the female openings in the XIII segment and not in the intersegmental line XII/XIII. In this genus however the ovaries lie in XII and the position of the last heart in X, instead of XI and IX respectively as is the case with *Drawida*. As to the number of segments occupied by the clitellum, the present species differs much from the other known species. X–XIII or four segments are most frequently noted in the exotic species while the Japanese species possesses as many as seven to eight segments occupying IX–XV or $\frac{1}{2}$VIII–$\frac{1}{2}$XVI. Some variations in this character so far noted with several other known species are as follows:

> *Drawida fluviatilis* occupies 5 and $\frac{1}{2}$ segments.
>
> *D.   grandis* occupies 3 and 5/6 segments.
>
> *D.   annadalei* occupies 5 and 1/3 segments.

Since the clitellum in *Drawida* is feebly developed and lacked by most of species which are sexually matured, we may consider that this variation can not be considered too seriousely for species determination especially when we remember that within the Japanese species many lack the clitellum entirely and furthermore the number of segments occupied is not constant.

It is interesting to mention that in this Japanese species the septa are thickened in V/VI–VIII/IX as with most other known species but in addition the Japanese species possesses another set of thickened septa in XVI/XVII–XIX/XX. The occurrence of thickenened septa in the anterior and again in a far behind region is mentioned with

no other species of *Drawida* but was noted with the two species of
Eupolygaster; *E. viridis* and *E. houteni* in which post-gizzard thickening
occurs at XIX/XXV and XVIII/XIX–XXIII/XXIV? respectively. In
this character the Japanese species again resembles Genus *Eupoly-
gaster* but differs from it in the position of the ovary, ovisac, and
last heart, which can be taken as more weighty taxonomic characters,
so we will be justfied in classifying this Japanese worm into *Drawida*
rather than into *Eupolygaster*. One may question whether or not
the thickness of the septa in the posterior region always drew much
attention in every species *Drawida* recorded in the literature.

The last point of difference is the microscopical size of the
nephridial pores. The nephridial duct which leads to the exterior
narrows suddenly and its caliber is no more thick than the nephro-
stome duct, while with the exotic species the duct leading to the
exterior rather enlarges and as the consequence the external pores
become very conspicuous.

This character just stated seems to be the most unique character
of our Japanese *Drawida* and requires further physiological inter-
pretation. Search through the literature fails to find any known
species which come closer to the present species even if we make
very generous allowance as to climatic, local and nutritional differences,
and I am forced to conclude that the *Drawida* species found in
Ishikawa prefecture is a new species which is not yet record. There
are however several reasons to suppose that the present worm is not
an endogenous species and its original home land may in future be
found to be elsewhere, most probably in some Eastern tropical lands.
Some of the reasons are as follows:

1. Despite the fact that practically all of the knowm species
belonging to this genus are strictly found in the tropics, this Japanese
Drawida is found in the region of Japan where winter is very severe
and even during the summer months the temperature is not nearly
so high as in the subtropical countries.

2. The worms are strictly localised to the villages near Kahoku
laka in Ishikawa prefecture. All attempts to find this species in other
localites of a likely producible area within a sphere of fifty miles
failes, and even in the next villages were unsuccessful.

3. In Hatta village the worms are so numerous that hundreds

may be gathered within one hour but the species found is only one.
I have examined a large number of smaller and larger worms with
melanic differences with or without papilla macroscopically and some
microscopically but failed to find worms with any deviation of im-
portance from the species here described-in this present report.

4. Strict localisation to a narrowly limited territory was accom-
plished by mutual agreement among the villagers concerned to prohibit '
importing the young rice plants even in case of failure of their rice
nursery beds, or of any other live plants in fear of the stowaway
journey of these worms with the soil attached to those plants. Such
observance of the regulation can not be imagined as having been
practiced during historically long ages.

5. A rather sudden appearance within a recent period seems
reasonable from various stories told concerning the origin of this
giant worm in Hatta village. Among the stories the following is one.
Years ago a young man of wealth was engaged to a poor girl of
the village but married another girl in the villa instead. The first
girl drowned herself from anger exclaiming that she would become
an earthworm and exterminate the young rice plants of the unfaithful
man. That was how this snaky and hairy worm become aboundant
only in this village.

This story seems to suggest that the worms appeared in abundance
rather suddenly to this village and such an appearance may not be
of too ancient origin, as there is no case of ancient tale or folk lore
in connection with the earthworms. We can imagine that a rather
sudden appearance in someone's rice field was explained by the
ignorant, superstitious villagers by the story given above.

My first expectation for the original home land of this species
was either India, Java or the Philippine islands from the reason that
this Hatta village, where this worm is found and localised, is famous
as the birth place of a hero merchant and explorer who lived there
about eightly years ago and who, according to popular belief, had
traded in person with those various countries.

This popular hero, GOHEI ZENIYA, had accumulated enormous
wealth from forbidden foreign trade, but unfortunately, he died in
1852 in a prison.

    GOHEI ZENIYA, the merchant and explorer, is said to have

brought many curios together with living plants in his big sailing ships. Naturally I attempted to trace the original habitates of this worm in those tropical countries, India,'Ceylon, the Philippines, Borneo, etc.

Unfortunately GOHEI ZENIYA left no record of his adventures, probably from fear of the discovery of his guilt which carried the death penalty at that time. Every effort has been made by historians in searching for even a bit of fact which would substantiate the popular belief in this foreign expedition, but they have not yet been rewarded.

At any rate, if the same species is found, or I am able to identify this Japanese species with any other known species living in the countries mentioned, my hypothesis is strengthened so that I may consider that the Japanese *Drawida* was accidentally transported by ZENIYA. Then this worm may furnish a key to solve this historical puzzle of long standing as to his whereabouts, but as was stated above the Japanese *Drawida* differs in too many important characters from the known exotic species and I am forced to consider it as a new species of *Drawida* which has not yet been found in other countries.

If this Japanese species was of insignificant size, we might then be reasonably assured that this species is still waiting for discovery. However its enormous body size and great fertility suggest that this species, if living in India where even very minute sized *Drawida* are named, could not have escaped the notice of zealous naturalists there.

If the species were actually imported, as I imagine, then the same species might be found in future in tropical lands other than India and more likely in the Philippine Islands or in Java where GOHEI ZENIYA is supposed to have visited, judging from his possession of a large quantity of calicos and numerous other articles characteristic of the countries mentioned.

Drawida japonica is the only other known species mentioned living in Japan but original paper of MICHAELSEN failed to mention its locality.

At any rate, the present writer awaits with great interest the rediscovery of *Drawida hattamimizu* somewhere else than in Japan, in other tropical lands, since there are still left much unexplored

territory for the *Drawidan* species in the Philippine Islands, hundreds
of South Sea Islands, Siam, Indo-China, etc. though not much of it
is left in India. Therefore, if the Japanese species were really trans-
ported from the tropics, as I imagine, then we may yet uncover its
real homeland in the future.

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On the Reproductive Process of the Earthworm, Pheretima communissima, (Goto et Hatai). Part I.

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(With Pl. XV and 3 Text-figures)

INTRODUCTION.

According to A. J. Grove (1925), the diverging views as to the reproductive processes of the Earthworm may be summarised as follows:

" (1) That during sexual congress the two worms are so placed that the clitellum of one is opposed to segments 9–11 of the other; that the discharge of spermatozoa is mutual, and that the seminal fluid is conducted along two grooves or furrows in each worm from the apertures of the vasa deferentia to the clitellum, where it accumulates about the apertures of the spermathecae of the other, and passes thence into them. (Hering, Andrews).

(2) That the seminal fluid passes along a tube formed by the adposed hollowed-out vessel surfaces of the two worms. (Cerfontaine).

(3) That the seminal fluid passes along two tubes formed by the adposition of grooves on the sides of each worm. This arrangement introduces the difficulty of simultaneous discharge, and infers that the discharge is not mutual. (Cole).

(4) Divergence of view as to the relative positions of the two worms."

And Grove himself has given firstly very careful and detailed accounts of the reproductive processes of Lumbricus. Then just subsequent to this, he and his co-worker L. F. Cowley (1926) again have studied the same subject in Eisenia foetida, and Karm Narayan Bahl in Eutyphoeus walotoni. So they have finally disposed of remarkable differences in the hitherto incorrectly given descriptions

upon the very subject in different reports and text-books.

In the reproductive processes of the Japanese Earthworm, *Phere-tima communissima* (GOTO et HATAI), the mode of exchange of the seminal fluid between two worms is entirely different from those described by GROVE and his co-worker and differ somewhat from the case of *Eutyphoeus*. In fact, the process is altogether more simple and direct than that of *Eutyphoeus*.

On account of the scarcity of any literature concerning the reproductive processes of the Japanese Earthworms, I have made these observations upon the phenomena

My thanks are due to Professor HATAI, whose encouragement and suggestions have been of great value to me.

METHODS

Under natural conditions, as stated by previous observers, *P. communissima* come to the surface to feed and for reproductive purposes, only between twilight and dawn, and as such conditions are not specially convenient for close study, an attempt was made to reproduce these conditions in the laboratory. The worms were placed in a large, flattened photographic bath that contained some 3 cm. of earth. This earth was usually brought from some place where worms were naturally abundant. The bath was kept in dark (photographic) room and the contents was kept moist and warm by means of pouring water and a wooden cover.

Though this was carefully done, the worms did not remain in a healthy, active condition; so it was found advisable to change the compost at least twice a month. Under the influence of the darkness and warmth, some of the worms were continuously on the surface, and several examples of sexual congress were observed and could be photographed.

Because of the worms' high sensitivity to any stimuli, such as light, moisture, etc., it was found that, though once connection was established, the worms did not become insensible to light, even to that of the red lamp, excepting very feeble red light. And, what is more, some of the worms would not tolerate even slight touching upon their body surface or shaking the bath itself. Therefore, some

close examinations of the conjugating worms were rendered very difficult, the photographic method was uniformly relied upon.

To take photographs of the pairing worms, the following steps were executed in order. 1) the cover of the bath was removed quietly with much care, 2) a peep of red light was thrown over the surface of earth in the bath, seeking for the pairing worms, 3) after catching sight of the pairing worms, a series of photographing manipulations and managements, such as focussing and preparing for the flash-light, etc., were finished within a few minutes, and then 4) a photograph was taken.

In order to get rid of any unnecessary delay of time, the camera was set on the top of a round stool whose height was constant, so that it was unnecessary to focus so often. There is no need of saying that, while the photographing operations were being attended to, there were no disturbing noises or the like. Of course, there were some cases in which the pairing worms seemed to be uninfluenced even by somewhat intense red light or by a slight touching upon the worms directly. To eliminate any noises (or otherwise an unsatisfactory result would come about), even for footsteps produced by the experimentor's approaching to the bath for observation, great care was paid.

The photographing operation was scarcely over when there could not be seen any pairing worms on the surface of the bath, and here existed an awful confusion among all the worms that were in the bath. A great many worms came out on the surface and went here and there, some jumping up and down in a peculiar manner, and others crawling away as rapidly as they could. Sometimes some of the worms would stray out from the bath, throwing themselves upon the floor and eagerly searching for their refuges. Whether this was caused by the force of the flashing light or the sound produced by the explosion of the flash-light powder, the writer did not test at all. It is reasonable, from those facts presented by foregoing investigators, to attribute its cause to the extreme intensity of light.

For the purpose of determining the duration-time of the sexual congress of the worms, several series of direct observations were performed ; and also, to determine from which pair, a proximal or a distal pair, of the spermathecae of one worm would be fulfilled with

the seminal fluid of its partner first of all. But for the phenomenon as just above quoted, a series of photographs of pairing worms could not be taken without much labour.

Many efforts were made to obtain preserved materials of the conjugating worms *in situ*, which had been successfully secured by GROVE and his co-worker and also by BAHL But in my case, owing to their mode of copulation and to their extreme sensitivity, any attempts at preventing the separation of the conjugating worms from each other were in vain. As pointed out by GROVE and BAHL, the writer did cut the anterior part of the worms off from the tail quickly, so as to prevent them from getting near into their burrows. Pouring over the pairing worms directly the hot fixing agency also gave no good results. Even when both processes of cutting off the tail and pouring the fixative upon the worms were finished in a few seconds, there was complete separation between the worms.

In preparing serial sections of the worms, no new methods were employed. A primary difficulty, that would confront any one who tried to obtain these serial sections, was able to be put aside through washing the intestinal canal with running water. And also this difficulty was surmounted by making a longitudinal dorso-median incision in each worm and removing the entire alimentary canal, if the disarrangements of the internal organs did not interfere with his particular study.

As fixing agency a 70%-alcohol-formol solution was employed. Serial sections were taken through the regions containing both sexual organs, and were stained with DELAFIELD's haematoxylin and eosin.

Together with these microscopical investigations, the macroscopical anatomy was also performed.

Brief Life History of *Pheretima communissima,* (GOTO et HATAI) in Sendai.

It is worthy of notice that the life-career of *P. communissima* does not last for one year round in Sendai. And its whole developmental history falls quite naturally into three general periods.

I) *Production of the germ cells.* — This includes the formation of the germ cells, oö- and spermatogenesis, parental copulation, and cocoon-formation.

II) *Embryonic and prehatching period.* — This extends from fertilization to the hatching out from the cocoon: in other words, a younger life in the cocoon-capsule. The phase of embryonic development seems to last for a relatively long time, to which more accurate observation must be paid, and the conclusion of this period is by no means marked. Generally, just subsequently to the embryonic period follows the prehatching period. At first, the chief systems and organs of the embryo become definitely laid down, and feeding on the albuminous substance stored up in the cocoon, the embryo persists in its development. Here the development is very slow and consists in the elaboration of the parts marked out during the preceeding period. In the more advanced embryonic stage, there are no pigments in the body-wall, and presumably it occurs that, simultaneously to the using up of the albuminous substance, the characteristic pigmentation of the Earthworm appears. The length and diameter of the worm, at the very time of coming out from its cocoon-capsule, are respectively 3 cm. and 1.5 mm.; the number of segments is almost equal to that of the fully grown one, and the body colour of it is somewhat brighter.

The hatching of the worm out of the cocoon-capsule commences at the end of December of the same year as the cocoon-formation, and persists until April. But the hatching out from the cocoon is most abundant from the beginning of March to towards the end of April.

III) *Immature period.* — This extends from the hatching out to sexual maturity. Very soon after the hatching out the worm begins to eat grains of humous earth and to grow. Up to the end of June, the length of the worm increases and the diameter swells rapidly day by day. And there are histological differentiation and the gradual appearance of adult characteristics, especially the clitellum.

Thus, all over the suburbs of Sendai, we can easily collect ' *clitellumed* ' mature worms about the middle of July.

Just parallel to the sexual maturity, *production of the germ cells* begins ; but this will be referred to the parental worm. The sexual life of the worm starts from the beginning of September and continues for nearly two months," until death. Of course, during that sexual life the cocoon formation is necessarily performed.

Towards the end of November we can not catch sight of *Pheretima*
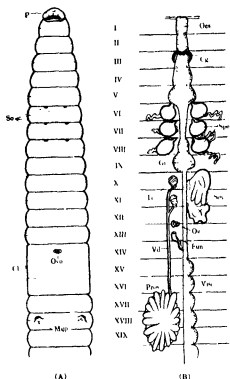
Fig 1   Figures show the general dispositions of the generative organs of *P. communissima* schematically   (A) The ventral view of the worm, showing the outer openings of the generative organs   (B) The inner dispositions of the generative organs, eliminating the right-side testes, vas deferens, prostate gland, ovary, oviduct, and also the left-side seminal vesicles and left half of the visceral canal beginning from the 10th segment posteriorly

P. Prostomium   *So* Spermatical opening.   *Ovo.* Opening of the oviduct.   *Mgp* Male genital papillae   *Oes* Oesophagus.   *Cg.* Cerebralganlion.   *Sper* Spermathecae. (including the ampulla and diverticula)   *Gi* Gizzard.   *Sev.* Seminal vesi le.   *Te* Testes.   *Vd* Vas deferens.   *Ov.* Ovary.   *Fun.* Ovidurtal funnel and oviduct.   *Prox.* Prostate gland   *Visc* Visceral canal.   *Cl* Clitellum.

communissima anywhere, though sometimes one or two entirely destroyed corpses may be dug out from some depth of ground.

The Generative Organs.
(Fig. 1.).

Since it is necessary to understand the disposition of the various parts to appreciate the details of the process of copulation and sperm exchange, a short description of the generative organs of *P. communissima* will be given here.

1) *Male organs.*—The male generative organs consist of (a) two pairs of testes with two pairs of seminal funnels, in the tenth and eleventh segments respectively, situated just prior to the distal pissenppiment of each segment; (b) two pairs of massive seminal vesicles, connected with the testes and funnels in the tenth and eleventh segments, but extending (distally) backwards and forwards so as to occupy in the adult worm segments 10,

11, 12, and 13 ; (c) a pair of vasa deferentia leading from the seminal
funnels down to the eighteenth segment, where there is a comparatively
wide, seminal atrium formed with its thick muscular wall ; (d) a pair
of penial processes on the ventral side of the eighteenth segment,
which act as copulatory organs ; and (e) a pair of dorso-ventrally
flattened prostates made of many folicle-like bodies, occupying eventually
segments 16, 17, 18, 19, 20, and 21. The disposition of all these
structures is illustrated in Fig. 1.

   2) *Female organs.* — The female generative organs consist of (a)
a pair of ovaries in the thirteenth segment, attaching to the distal
surface of the disseptiment 12/13 ; (b) a pair of oviductal funnels in
the same segment leading into the oviducts, which open to the ex-
terior on the fourteenth segment by one pore lying anterior to the
zone of the setae , and (c) three pairs of spermathecae in segments,
6, 7, and 8, and their openings intersegmentally on 5/6, 6/7, and 7/8.
Of these, the spermathecae are the only structures of interest in
studying the process of copulation in the worm. There are three
pairs of them and each consists of a thin-walled, sac-like ampulla and
a somewhat twitched, tube-like diverticulum whose wall is far more
thick than that of the ampulla. Each member of the spermathecae
opens with its own duct, which finally unites into, seemingly one
pore.

Direct Observaton upon the Copulating Worms.
(Pl. XV. Figs. A. B. C. D. E. F.).

   Two worms coming out of adjacent burrows or positions meet,
searching for their partners with their especially sensible prostomia,
and in less than five minutes get aposed to each other in the typical
head-to-tail position with their ventral sides applied to one another, as
shown in the accompanying plates. The process of searching for their
partners is very peculiar. Some 3 or 4 segments of the head parts
of each worm show a swinging motion, eagerly looking for their
partners' male genital papillae. To come to the most suitable position
the worms proceed and retire now and then, until the male genital
papillae on the eighteenth segment of each worm come to lie opposite
to the spermathecal pores (7/8) of the other. Regularly, the insertion
of the male genital papillae into the pair of the spermathecae that

opens between the seventh and eighth segments intersegmentally, takes place at first; and then next the anterior pair. By drawing in the ventral side, before the insertion of the genital papillae has occurred, each worm hollows out the ventral surface of the eighteenth segments and its neighboring segments containing the clitellum into a characteristic 'boat-shaped' depression (kahnformige Grube, HERING. 1857). The other worm lies in this excavation. Whilst, as described above, the ventral body-wall of the adjoining segments of the male pores shows a characteristic 'boat-shaped' depression, there is no indication of deformation in any segments of the co-operating worm, where the spermathecal pores open. But it may be depended upon that the absence of such deformation has much to do with the anatomical and physiological points.

The ampulla of the spermathecae is only a thin-walled sac which has no ability of self-contraction. In the course of copulation the ejaculatory atrium of the vas deferens squeezes out its content into the space of the ampulla, the latter only being a reservoir of the seminal fluid ejaculated by the former. Because of its passive function it might be very inconvenient, if the cavity which should be occupied by the whole spermathecae in the body-cavity of the worm would have been limited. Fortunately for the worm, on nineth segment there is a muscular gizzard that has a concrete form, and may probably act as a sustainer to hold out the body-cavity, much re-enforced by other organs and the body-wall itself.

While the ventral surfaces of the pairing worms are in such close contact, a very intimate connection is established between the region of the male pores of the one and the spermathecal pores of the other. As intimately as is established the connection of the two worms, there are neither the slime-tube (as secréted by Lumbricus or Eisenia) nor the paste-like slime which was observed by BAHL on Eutyphoeus. Despite this fact, we can perceive in Pl. XV. Figs. A. & E. something like the slime on the ventral side of one worm. On the other hand, Pl. XV. Figs. D. & F. shows no indication of the slime secretion. These indicate a seeming contradiction. Careful examination shows, however, that this slimy substance is no more than the usually secreted slime that keeps moisture upon the body surface of the worm. As has been mentioned above, the flash-light astonished the worms and

made them separate from each other all at once, leaving a short thread
of slimy substance between the two worms. And this thread-like
feature of slimy substance was exaggerated by the strong shades given
by the flash light.

Next, the colouration of the conjugating worms must be referred
to here. When the worms are in sexual congress, the colour of the
ventral sides of them becomes more reddish than usual, with no ex-
ception. So characteristic is this changing of colouration that one
can be sure, whenever and wherever he sees the phenomena on the
worms, that they are in sexual congress. Those photographs presented
in this paper show the phenomena with a gradation of whitish shading,
which will not exist when the characteristic colouration is too slight
and faint. (Pl. XV, Fig. C. and Fig. E.)

HERING (1857) is the first investigator, who determined the dura-
tion time of the sexual congress in *Lumbricus*, and the duration
would cover two or three hours. BRETSCHER (1901) reviewed all
observations of previous authors, saying HERING's determination might
be relied upon. GROVE (1925) reexamined the duration time as to
the same species again and got the same result But, according to
BAHL (1927), in *Eutyphoeus* the duration did not exceed one hour,
probably because ' the time taken for preliminary adjustment is very
small — less than five minutes.'

Indeed, in *Pheretima communissima*, the time taken for preliminary
adjustment is very small — less than five minutes; but four or five
hours were estimated for the duration time, and generally for filling
each pair of the spermathecae on the same segment, it took about
1.5 hour at least, as the following protocol shows.

Protocol. (20th. Oct. 1929.)

3 o'clock p. m.

          43 minute. . .catch sight of newly copulating worms,
                       and at every 10 minute the are ob-
                       served.

4 o'clock p. m.

           . . . . .the same as before.

5 o'clock p. m.

          20 minute. . .male processes are inserted into the
                       middle pair of the spermathecae.

6 o'clock p. m.

               50 minute. .male processes are inserted into the foremost pair of the spermathecae.

7 o'clock p. m.

            . . . . .the same as before.

8 o'clock p. m.

              20 minute. . .the pairing worms retire into their own burrows.

It is noticeable that in most cases (excepting this) the time which is taken for the middle pair of the spermathecae to fill themselves with the seminal fluid, is the longest; the reason could not be determined.

It was true that, while in copulation, the anterior five to seven segments of the co-operating worms kept free; but on account of this, there was, in *P. communissima*, no such case as described by BAHL, "Feeding and copulation therefore go on side by side at the same-time." Although sometimes, when the photographing manipulations were being prepared for, one or both of the pairing worms seemed to search for something with their prostomia, this does not correspond to " Feeding and copulation go on side by side." Only the sensible worms felt the unusual conditions, given rise to through the experimentor's managing actions or the red light, and then reacted timidly with inquiring manners. So if there were no such disturbing events, the worms were always still. (Pl. XV, Fig. F.).

The copulation of *P. communissima* is performed in every place, on the surface, in the burrows of the worm, or anywhere else. And, as has been said by many authors, they copulate between twilight and dawn, although their conjugation is seen even in day time if their 'population ratio' is relatively large.

The Modes of Attachment and Some Anatomical Facts.

Already, a general explanation of the mode of attachment of the copulating worms has been given from my direct observations, but there are several details that need to be described fully here.

BAHL (1927), with his direct observations upon the living pairing worms and the preserved hardened specimens, states, "There is no doubt that the most intimate connection between the worms is es-

tablished *at four places*, where 'peg and socket' joints are formed"
Looking, however, at the living conjugating worms solely, I cannot
help noticing that there exists no such intimate connection as can be
indicated by the expression, 'peg and socket' joints. Examination
of Pl. XV. clearly shows that the male genital papillae are simply
fitted into the spermathecal pores of the co-operating worm. And
the attachment is made somewhat more secure by the lying of one
worm in the excavation of the partner, and reversely. (Pl. XV, Fig.
A and A').

At ordinary times, the male genital papillae are not in the pro-
truded condition, in which there are seen in the sexual congress and
in some fixatives. Therefore when the worms are going to copulate
the male papillae must be protruded so as to fit into the spermathecal
pores This change from the normal disposition of the male genital
papillae into their copulatory disposition is brought about by the
action of the muscles surrounding the genital pit. The disposition of
the muscles surrounding the male genital openings has been determined
by a study of serial sections through this structure, and also by
a dissection of these muscles in hardened specimens. It has been
found that besides the circular and longitudinal muscles, which are
utterly continual to those of the body wall, there is another set of
muscles in the genital area of *P. communissima* which aids in copulation.

The contraction of the circular muscles and the elongation of the
longitudinal muscle simultaneously render the male genital papillae
protruded : a mechanism, which resembles the manner exhibited by
the anterior part of the worm itself when it is in proceeding motion.

The Arciform Muscles (*muscles arciformes,*
CERFONTAINE, 1890.)

Here another set of muscles, named 'arciform muscles', will be
referred to. They are ventro-lateral muscles in each of the segments
14 to 25, intrasegmentally lying on the inner coelomic side of the
body-wall. One end of each muscle terminates in the ventral median
line (*Bauchlinie*, VEJDOVSKY; *'le ligne median*, CERFONTAINE), which
is represented by a special longitudinal muscle-band ; and the other
end inserts into the longitudinal muscles of the somewhat dorso-lateral

body-wall, reaching finally to the epidermal layer through the circular muscle layer. (Fig. 2.) In the normal position, the body-wall stretches these arciform muscles across two points of their attachments, and under no circumstances is there any case in which the arciform muscles run alongside the body-wall. So, for example, they go over or sometimes pass through the prostate glands, when the glands are so massive. The length of each pair of these muscles is much divergent, and this affords a very worthy efficiency to the worm which is going to copulate; that is, the 'boat-shaped' depressions on the ventral surfaces of the pairing worms are invariably affected by the arciform
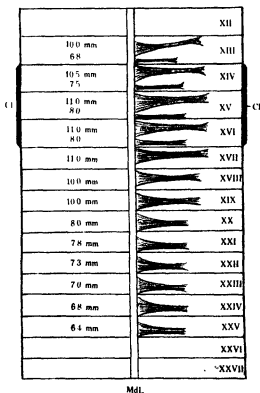
Text-fig 2. A right-half set of the arciform muscles with their respective numerical lengths. (shematized)
Cl. Clitellum. MdL Ventral median line. Am. Arciform muscles.

muscles. The reason is as follows: the longer the arciform muscles are, the nearer dorso-laterally the one end of the muscles can get to the body-wall; and this fact involves in itself that, where there is the longest muscle in one segment, there must be manifested the deepest excavation on the segment, drawing the ventral body-wall back into the body-cavity. And thus, acting their respective roles as fully as can be allowed, the arciform muscles hollow out the 'boot-shaped' depression on the ventral surfaces of the pairing worms within a relatively short time, and the anterior part of each worm containing the spermathecal openings is just adposed to the boat-shaped depression then constructed. In Pl. XV. we see depressions of irregular shape superficially on the region near the root of the male genital papillae. These depressions are formed by the force of the contraction of the arciform muscles. BAHL (1927) has called attention to this muscle, naming it 'the special ventro-lateral muscle', although he has not explained its function sufficiently. Comparing his text-figure, which gives a semidiagramatic representation of the muscle, with mine, I find the dispositions of these muscles are decidedly different; that is, BAHL's is intersegment and mine intrisegment.

As to the histological investigations of the arciform muscles, a subsequent paper will be issued presently.
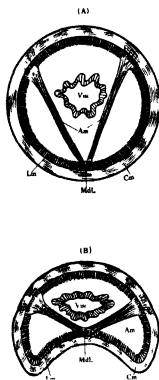
Text-fig 3 Figures show the mode of attaching of the arciform muscles, and their manner in action shematically. Cross-section of the worm by seventeenth segment (A) Normal uncontracted state of muscles. (B) Contracted state of muscles, showing a peculier cross-section.
Am Arciform muscles   Lm. & Cm. Longitudinal and circular muscles of the body-wall   MdL. Ventral median line   Visc. Visceral canal.

The contribution which needs be considered here is that of CERFON-TAINE (1890), who, in describing four kinds of muscles in the body of the Earthworm, defines the ' muscles arciformes' (pp. 407–408). According to CERFONTAINE, in Lumbricus and also in Eisenia his ' muscles arciformes' are serviceable to cause a boat-shaped depression on one hand and ' bourrelets du sillon génital' on the other hand. Since there is no trace of the structure called ' bourrelets du sillon génital' in P. communissima, it is by no means too bold to call the muscles, ' arciform muscles' (muscles arciformes).

SUMMARY

1. As there are relatively few literatures concerning the repro-ductive processes of the Japanese Earthworms, I intend to show the phenomena more clearly and exactly.

2. For what reason I had to employ solely the photographic method, is explained and the accessory methods of investigations are fully given.

3. The life-history of P. communissima (GOTO et HATAI) in Sendai is determined, and its general periods are settled.

4. Full description of the reproductive processes of P. communis-sima are given. And it is found that the processes are entirely differs from those of Lumbricus and Eisenia, and somewhat from those of Indian Earthworm, Eutyphoeus.

5. The duration-time of the copulation of the Japanese Earthworm is determined through direct observations; and four to five hours are estimated for it.

6. The modes of attachment of the pairing worms and some anatomical facts, especially of the ' arciform muscles' are described.

7. The observations of the cocoon-formation are not able to complete.

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EXPLANATION OF PLATE XV.

These photographs will represent really the natural manners of the sexual connection of the Earthworm, because, as has been already explained in the text, the copulating worms were neither touched at all nor were dug out so as to make them come to the surface of earth Except Fig. A & A′, the photgraphs are printed directly from negative plates.

Fig. A & A′ show how the male genital papillae of one worm are inserted into the spermathecal openings of the other and how the body-wall of the root of the male genital papillae are hollowed out by the force of the arciform muscles. These photographs are produced by enlarging two parts of Fig. B. Such enlarging will afford us more exact knowledge about the mode of copulation. Something like the slime is seen between the ventral side of the both worms. It is nothing but the usually secreted slimy substance that keeps merely the moisture upon the body surface of the worm And it happens that this slimy substance reflects so strongly the flash light that we can conceive it very conspicuously.

　　　　Sp......Spermathecal opening　　　Mgp......Male genital papilla.
　　　　Cl....Clitellum.

Fig. B.　Photographed on 9th. Oct. 1929. Only the anterior parts of the body are exposed to the free surface. The first photograph of this observation. ×1.

Fig. C　Photographed on 13th. Oct 1929. As has been quoted in the text, 'When the worms are in sexual congress, the colour of the ventral sides of them becomes more reddish than usual with no exception.' Such characteristic changing of colouration is represented in this photograph with a gradation of whitish shading. (×1).

Fig. D.　Photographed on 20th. Oct. 1929. In this photograph the slimy substance is not seen any more. (×1.5).

Fig. E.　Photographed on 16th. Oct 1929. One of the copulating worms exposes entirely its body, while the other makes its posterior part still in earth. (×4/5).

Fig. F.　Photographed on 31th. Oct. 1929　Relative position of the posterior parts of the copulating worms will be seen clearly. As the anterior five segments of the copulating worms kept free, so in the photograph one of them make its anterior part uplifted, reacting to some unusual condition with timid, inquiring motion. (×1).

Report of the Biological Survey of Mutsu Bay.

16. Macrura of Mutsu Bay.[1]

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(With Plate XVI and 5 text-figures.)

The Macruran specimens collected by the survey amount to over 400 in number representing 13 genera and 23 species. Among them, one genus, *Paraspirontocaris* and four or probably five species are new to science; i. e., *Paraspirontocaris kishinouyei*, *Spirontocaris minuta*, *Sp. japonica*, *Gebia affinis* and *Pandalus sp.*

By the study of the collection, we can learn many interesting facts, especially from the view point of the geographical distribution. For instance, *Penaeus japonicus* which has not hitherto been known north of Akita-prefecture on the side of the Japan Sea and whose northern limit of distribution on the Pacific side was probably the Cape Inuboe-zaki, is now found to exist even in a fully grown stage so far north in the bay. It is also ascertained that such forms as *Erythropenaeus akayebi* (RATHBUN), *Alpheus japonicus* MIERS, *A. distinguendus* DE MAN, *Latreutes laminirostris* ORTMANN, *Leander serrifer* STIMPSON and *Gebia major* DE HAAN which are all rather southern inhabitants similarly have their existence in the bay. On the other hand, it is interesting that the northern form, *Spirontocaris prionota* which has already been recorded far from California and Behring Sea is again here collected; and concerning the new species, such as *Spirontocaris minuta*, *Sp. japonica* and *Pandalus sp.*, their nearly allied forms have been found from Behring Sea, North America or somewhere else.

Finally, it is with great regret that I call attention to the death of Prof. K. KISHINOUYE, who promised to cooperate with me in the present study.

Tribe PENAEIDEA.

Family **Penaeidae** BATE.

Genus PENAEUS BABRICIUS.

1. **Penaeus japonicus** (BATE).

Penaeus canaliculatus var. japonicus BATE., 1888, p. 245, Pls. 31, 32, 37.
Pei aeus japonicus, DE MAN, 1911 (Siboga), p 107, (other previous literature),
    BALSS, 1914, p 13

Japanese name: Kurumayebi.

Loc. Moura. July 19, 1926 2 males.

    Off Gomijima. August 16, 1926. 1 female.

The female specimen is 21 cm. long and 85 gr. in weight.

General Distribution: Indo-pacific region In Japan, it has been well known from Kiushû, Shikoku and Honshû, but on the coast of the Japan Sea it has not been known north of Akita Prefecture and on the Pacific side never known north of the Cape Inuboe-zaki.

Genus TRACHYPENAEUS ALCOCK.

2. *Trachypenaeus curvirostris* (STIMPSON).

Penaeus curvirostris STIMPSON, 1860, p 44, DE MAN, 1907, p 436
Parapenaeus curvirostris RATHBUN. 1902, p 38,
Trachypenaeus curvirostris, BALSS, 1914, p. 11.

Japanese name: Saruyebi.

Loc. Off Nonai, August 18, 1925. 1 female.

    Futago-ôshima. July 30, 1926. 2 males and 3 females.

    Okunai. July 31, 1926. 1 female

    Off Futatsuya. July 24, 1927. 3 females.

General Distribution: Arafura sea. Japan: Kiushû, Shikoku, Honshû, south of Corea.

Genus ERYTHROPENAEUS KISHINOUYE.[1]

3. **Erythropenaeus akayebi** (RÀTHBUM).

Parapenaeus akayebi RATHBUN, 1902, p. 39.

Japanese name: Akayebi.

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[1] KISHINOUYE, 1929, p 283.

Loc. Off Tsubakiyama. July 24, 1927. 1 male and 1 female.

General Distribution: Japan: Kiushû, Inland sea. Occurrence in Mutsu Bay is interesting.

Genus CERATOPENAEUS KISHINOUYE.[1]

4. Ceratopenaeus dalei (RATHBUN).

Parapenaeus dalei RATHBUN, 1902, p. 42.

Loc. Between Moura and Namiuchi. July 12, 1926. 1 male and 1 female.

    Okunai. July 31, 1926. 2 females.

    Off Shirasu. August 1, 1926. 1 female.

    Off Kawauchi. August 11, 1926. 1 male.

    Off Noheji. August 22, 1926. 1 male.

    Off Tairadate. July 24, 1927. 1 male and 1 female.

    Off Higashiokuyakata. August 10, 1927. 2 males.

General Distribution: It has hitherto been recorded from Moji and Hakodate, Hokkaido.

Tribe EUCYPHIDEA.

Family Alpheidae BATE.

Genus ALPHEUS FABRICIUS.

5. Alpheus japonicus MIERS.

Alpheus japonicus MIERS, 1879, p 53, ORTMANN, 1891, p 476, pl 36, Fig 14.

Japanese name : Tenagateppô-yebi.

Loc. Off Moura. 12–13 fms. Sandy mud. July 20, 1926. 1 egg-bearing female.

    Between Moura-kojima and Futagojima. 12,5 fms. Sandy mud. July 21, 1926. 1 male.

    On the line between Ôshima Isl. and Aomori, off the Marine Biological Station, Asamushi. 1 egg-bearing female.

    On the line between Cape Futagozaki and Ôshima Isl., off Cape Aburamezaki. 27 fms. Sandy mud. July 30, 1926. 1 egg-bearing female.

[1]KISHINOUYE, 1929, p 282.

Between Tsuchiya and Moura. August 10, 1925. 2 males and 3 egg-bearing females.

Off Futatsuya. July 24, 1927. 1 male.

General Distribution: Southern Japan: Tokyo Bay, Tanagawa, Yokosuka, Kobe.

6. Alpheus distinguendus DE MAN.

Alpheus rapax DE HAAN, 1849, p. 177, Pl 45, Fig. 2; BATE, 1888, p. 552, Pl. 99, Fig. 1, DE MAN, 1888, p 284, ORTMANN. 1891, p. 481.

Alpheus distinguendus DE MAN, 1909, p 155, Pl. 7, Figs. 9–14.

Loc. Off Aomori. 14–15 fms. Bottom mud. June 1, 1926. 1 male.

General Distribution: Japan; China; Mergui-Archipelago.

Family Hippolytidae ORTMANN.

Genus LATREUTES STIMPSON.

7. Latreutes laminirostris ORTMANN.

Latreutes laminirostris ORTMANN, 1891, p 506, Pl. 37, Fig. 5, DE MAN, 1907, p. 422.

Loc. Moura. 5 fms. Sand and sea-weeds. July 20, 1926. 1 male and 1 egg-bearing female.

Ôma Bay. August 18, 1927. 1 male.

General Distribution: Japan: Tanagawa, Inland Sea.

Genus SPIRONTOCARIS BATE.

8. Spirontocaris prionota (STIMPSON).

Hippolyte prionota STIMPSON, 1864. p. 153.

Spirontocaris prionota, BATE, 1914, p. 42: SCHMITT, 1921, p. 52, Text-fig. 28.

Loc. Off Arito. 19 fms. Sandy mud. August 22, 1926. 1 male.

General Distribution: From Behring Sea to Monterey, California; Japan: Aomori.

9. Spirontocaris pectinifera (STIMPSON).

Hippolyte pectinifera, STIMPSON, 1860, p. 35

Spirontocaris pectinifera, BATES, 1914, p. 42, Text-figs. 23, 24.

Loc. On the line between Futagojima and Cape Hanaguri-zaki,

off Namiuchi. Sandy mud. August 5, 1926. 1 male.

  General Distribution: Japan: Hakodate, Dsushi, Negishi near Yokohama.

10. Spirontocaris mororani RATHBUN.
(Text-fig 1).

Spirontocaris mororani RATHBUN, 1902, p. 43, Text-fig. 16

  Loc. Between Yunoshima Isl. and Asamushi. 5–6 fms. Sea-weeds.
    August 19, 1926. 3 males.
    About 1500 metres off Jôgasawa. 11 fms. Sea-weeds. August
    18, 1926. 1 female.
    Off Noheji. 5 fms. Sea-weeds. August 22, 1926. 1 male.
    Off Arito. 19 fms. Sea-weeds. August 22, 1926. 1 male.
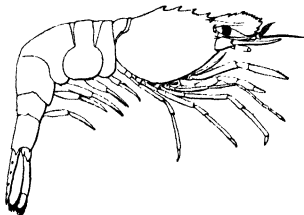
Text-fig. 1. *Spirontocaris mororani* RATHBUN. (×4).

  These specimens are all smaller than the type specimen described by RATHBUN; the largest one from between Yunoshima Isl. and Asamushi is 24.2 mm. long from the tip of the rostrum to the end of the telson.

  The rostrum is shorter than that of the type specimen and scarcely longer than one half the length of the rest of the carapace, and its tip is similar in feature to that of *Spir. ochotensis* (BRANDT).* It

* BRANDT, 1851, p. 120, Pl. 5, Fig. 17.

exceeds a little the distal end of the peduncle of the first antenna.
Four prominent teeth are on the dorsal carina of the carapace. Of
the two supraorbital spines the anterior is distinctly smaller than the
posterior.

    General Distribution: Muroran, Hokkaidô.

11. Spirontocaris pandaloides (STIMPSON).

Hippolyte pandaloides STIMPSON, 1860, p 34; DOFLEIN, 1902, p 637, Pl 5, Fig. 3
Spirontocaris pandaloides, DE MAN, 1907, p 418, Pl 32, Figs 47, 48.

Japanese name: Tunonagamoyebi.

Loc. Between Yunoshima Isl. and Asamushi. 5–6 fms. Sea-weeds.
    April 29, 1926. Numerous specimens of both sexes.
    Off the mouth of the Shimizugawa. 17 fms Sandy mud. July
    4, 1926 Many specimens.
    Moura. 5 fms. Sea-weeds. July 20, 1926. Many specimens.
    About 1500 metres off Sumichigai. 9 fms. Sea-weeds. August
    11, 1926. Many specimens.
    Off Noheji. 5 fms. Sea-weeds. August 22, 1926. 6 males and
    2 females.
    Ôma Bay. August 18, 1927. 1 male.
Rostrum with 7–10 teeth above, 9–12 below.
    General Distribution: Hakodate to Inland Sea of Japan ; Corea
Strait.

12. Spirontocaris geniculata (STIMPSON).

Hippolyte geniculata STIMPSON, 1860, p 34, ORTMANN, 1891, p. 503, Pl. 37, Fig. 3.
Spirontocaris geniculata, RATHBUN, 1902, p. 45, Text-fig 19.
Spirontocaris alcimede DE MAN, 1907, p 416, Pl. 32, Figs. 42–46.

Japanese name: Kosimagarimoyebi, Kusakosiyebi.

Loc. Between Yunoshima Isl. and Asamushi. 5–6 fms. Sea-weeds.
    Many specimens of both sexes.
    do. August 23, 1927. 2 immatures.
    Moura. 5 fms. Sand and sea-weeds. July 20, 1926. 2 males,
    and 1 immature.
    Off Sumichigai. 9 fms. Sea-weeds. August 11, 1926. 6 males
    and 6 females.

Off Noheji. 5 fms. Sea-weeds. August 22, 1926. 3 males and
1 female.

Off Arito. 19 fms. Sandy mud. August 22, 1926. 1 male.

The coast of Tsuchiya, among sea-weeds. August 23, 1926.
7 males.

The mandible in the specimens here examined, is furnished with
a palp in two segments, while in the figure given by ORTMANN the
palp seems to be three segmented. Some specimens are armed with
a minute branchiostegal tooth on either or on both sides. Rostrum
with 5-7 teeth above, 6-7 below. The external maxilliped is provided
with a rudimentary epipodite in the present specimens, but in other
respects it agrees with the description of *Spirontocaris alcimede* DE
MAN.

On the supposition that DE MAN overlooked the epipodite in his
Spirontocaris alcimede his name becomes a synonym of *S. geniculata*
(STIMPSON).

General Distribution : Japan: Hakodate, Muroran, Tanagawa,
Tokyo Bay, Inland Sea.

13. Spirontocaris rectirostris (STIMPSON)

Hippolyte rectirostris STIMPSON, 1860, p. 33; DOFLIN, 1902, p. 637, Pl 3, Fig 7

Spirontocaris rectirostris, DE MAN, 1907, p 411, Pl '32, Figs 31-34, BALSS, 1914,
p 43

Japanese name : Asinagamoyebi.

Loc. Between Yunoshima Isl. and Asamushi. 5-6 fms. Sea-weeds.
April 29, 1926. 18 egg-bearing females.

In front of Benten, Yunoshima Isl. January 18, 1927. 11
females.

Off Kanita. July 23, 1927. 1 male and 2 females, of which
one bore eggs.

Rostrum with 5-7 teeth above, 3 or 4, very rarely 2, teeth below.

General Distribution : Japan: Hakodate, Aomori, Sagami Bay,
Inland Sea, Nagasaki.

14. Spirontocaris minuta, n. sp.
(Text-fig 2)

Loc. Off Arito. 19 fms. Sandy mud. August 22, 1926. 1 specimen,

probably male.

Closely related to *Spirontocaris decora* RATHBUN[1] from Pacific Coast of North America, but different from this in the longer rostrum and its feature and denticulation; the sixth abdominal segment is relatively shorter than that of the American species.
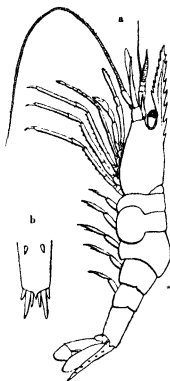
Text-fig. 2. *Spirontocaris minuta*, n. sp.

a. Entire animal, view from left side (×4).

b. Terminal half of the telson, dorsal aspect.

This specimen is 23 mm. long from the tip of the rostrum to the end of the telson; the abdomen, which is moderately geniculated at the third segment, is almost one and a half times as long as the carapace (rostrum included). The free part of the rostrum is one and a third times as long as the rest of the carapace; it arises as an obtuse crest at a half length of the cephalothorax from its anterior border; it projects horizontally, and is armed with seven teeth above and below. These teeth are all subequal in size and almost equidistant, but the anterior one on the upper margin stands nearer to the next. The posterior one is behind the posterior margin of the orbit.

There is no supraorbital tooth, but an antennal and a branchiostegal tooth are present; both of them are moderate in size. The abdomen is laterally compressed and rounded above. The fourth abdominal segment is distinctly longer than the fifth, and its epimeron is rounded at the posterior angle, while in the fifth it is pointed to a sharp tooth; the sixth segment, which is a

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[1] RATHBUN, 1902. p. 896.

little longer than twice the length of the fifth, is almost twice as long as broad; its postero-lateral angle terminates in a sharp point. The telson, which is about one and a third times as long as the sixth segment and almost equal in length to the uropods, terminates in a triangular point and three pairs of spinules. Of these spinules, the outer one is the shortest and the middle is the longest, while the inner one is intermediate between the two. The dorsal side of the telson is armed with five pairs of spinules.

The eye-stalk is of moderate size and the distal end of the cornea scarcely reaches to the base of the third tooth on the upper margin of the rostrum. The first pair of antennae exceed the tip of the rostrum with a part of the inner flagellum, while the outer flagellum is shorter and stouter than the inner and does not reach the extremity of the rostrum. In the peduncular segments, the first is the longest and the succeeding two segments are shorter than the first, and each of them is armed with a sharply pointed spine at the external extremity. The stylocerite is terminally pointed and reaches the extremity of the first peduncular segment. In the second pair of antennae the basal segment is terminally produced to a sharp point, and the scaphocerite scarcely attains to the level of the rostral extremity; the flagellum is about as long as the body-length without the rostrum. The external maxilliped is furnished with a rudimentary epipodite, but not with an exopodite. In the pereiopods the anterior three pairs are provided with epipodites. The carpus of the second pair is divided into seven articles, in which the second is the shortest and the third is the longest. The posterior three pairs of legs are similar in feature, and their meri and carpi are provided with series of spinules on their posterior borders.

15. Spirontocaris japonica, n. sp.

(Text-fig. 3).

Loc. Between Yunoshima Isl. and Asamushi. 5–6 fms. Sea-weeds. April 29, 1926. 4 young specimens.

These specimens are of small size, the largest of which is 19,4 mm. and the smallest 13,5 mm. in total length from the tip of the rostrum to the end of the telson. The rostrum is armed with four teeth above on the proximal half, while the distal half of the rostrum is devoid

of teeth. These four teeth are subequal in size and the posterior one is behind the posterior margin of the orbit. The length of the rostrum is variable according to the size of the specimen; in the smallest specimen the rostrum is very slender in shape and reaches scarcely to the tip of the basal segment of the first antenna, while in the largest one it is much deeper than that of the former and reaches far beyond the extremity of the peduncle of the first antenna, attaining to the extremity of the scaphocerite of the second antenna. There is no supraorbital tooth; the outer angle of the orbital margin terminates in a rounded tooth or lobe, and an antennal and a branchiostegal tooth are usually furnished on the anterior portion of the carapace. Of these two teeth the former is larger than the latter; the latter is sometimes obscure. The eye-stalk is rather large and pyriform. The distal end of the eye reaches to the anterior tooth of the upper margin of the rostrum. In the first pair of antennae, the stylocerite is terminally pointed and shorter than the basal segment of the

Text-fig. 3. *Spirontocaris japonica*, n. sp.
a. Entire animal, view from left side (×6).
b. Mandible.

peduncle, which is very long, especially in smaller specimens. In the peduncular segments the proximate is the longest and the others are much shorter than that; each of the segments of the peduncle is terminally armed with a spinule on the outer border.

The outer maxilliped, reaching to the level of the end of the basal peduncular segment the first antenna or a little beyond it, is furnished with an exopodite and an epipodite. The anterior three pairs of legs are also provided with epipodites. The abdomen, which is smooth surfaced and dorsally rounded, is geniculated at the third segment, where it is unarmed. The sixth abdominal segment is about as long as twice the length of the fifth, and a little shorter than the telson. The pleura of the fourth and the fifth abdominal segments are pointed posteriorly. The telson, which carries four pairs of spinules on the dorsal surface, tapers gradually posteriorly and its posterior margin ends in the middle in a sharp tooth, and of the two spines on either side the outer is a little shorter than the inner.

The species is allied with *Spironto. fabricii* (KRÖYER)[1] and *Spironto. middendorffii* BRASHNIKOW.[2] From the former it differs in the following points. The rostrum is much longer than the length of the rest of the carapace, and most of the teeth on the upper margin of the rostrum are in front of the posterior orbital margin, These differences, in my opinion, are too distinct to attribute to their immaturity. From the latter it differs in the toothing of the rostrum and in the absence of the abdominal armature.

Genus PARASPIRONTOCARIS, n. gen.

The surface of the body is rather uneven and covered with short hairs. The rostrum projects obliquely downwards, and is laterally compressed; a rib on each side is well developed and the upper margin is provided with small teeth, which are rather conical in shape and directed nearly perpendicular to the margin. The supraorbital tooth is strong and the branchiostegal tooth is also well developed, while the antennal is wanting. In the abdominal segments a half of each of the first and the fifth segments is dorsally carinated in the

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[1] RATHBUN, 1929, p. 15, Text-fig. 15.
[2] BRASHNIKOW, 1907, p. 168, Figs. 23 a–b.

medial line and the other half is provided with a paired carinae, while in the second, the third and the fourth segments a strong carina is on the medial line. The sixth and the seventh segments are dorsally rounded, but the former projects posteriorly in three points. The latter or the telson is provided with some minute spinules.

The eye-stalk is moderate in size. The first antenna is provided with a well developed stylocerite and bears two flagella, a stout and a slender one. The mandible is divided into two portions and provided with a palp in two segments. The second maxilliped bear a rudimentary mastigobranchia, and the podobranchia is obscurely developed. The external or third maxilliped is four segmented and devoid of the exopodite. The anterior two pairs of the pereiopods are chelate, and the carpos of the second is subdivided into seven articles.

The branchial formula is as follows : —

	h	i	k	l	m	n	o
Pleurobranchiae	- -	1	1	1	1	1	—
Arthrobranchiae	—	—	—	—	—	—	—
Podobranchiae	r ?	—	—	—	—	—	—
Mastigobranchiae	r	r	r	r	r	—	—

The new genus is nearly allied with the genus *Spirontocaris,* but differs in its peculiar shape and the armature of the rostrum, in the paired carinations of the first and the fifth abdominal segments, and in the branchial formula. The spinules of the telson, in this genus, are very obscure under the naked eye.

15. Paraspirontocaris kishinouyei, n. sp.
(Pl XVI, figs. 1–6)

Loc. On the line between Kanita and Yokohama, off Ôshima Isl. July 17, 1927. 1 male.

Off Tairadate. July 24, 1927. 1 male.

The specimens are 27,9 and 22,5 mm. in length, measuring from the tip of the rostrum to the end of the telson. The body is rather stout and covered with short hairs. The carapace including the rostrum is about one and a half times as long as the abdomen, and dorsally carinated in the medial line. This dorsal carina of the carapace is armed with two small tubercles and is slightly notched

above the base of the supraorbital tooth. The rostrum, which projects obliquely downwards, is not only laterally compressed but also provided with a rather strong rib on each side and is a little longer than two thirds the length of the rest of the carapace. On its superior margin it is armed with minute teeth which are rather conical in shape and stand at right angles with the margin. At the base of the rostrum there is a very strong supraorbital tooth on each side, directing forwards and outwards. At the branchiostegal angle the carapace is dilated outwards and forwards to a sharp point, but is devoid of any antennal tooth.

The eyes are moderate in size. The first pair of the antennae exceeds the distal end of the rostrum with a part of the flagella, in which the outer is much thicker than the inner; in the peduncular segments the proximal is longer than the succeeding two segments together. The stylocerite is well developed, almost reaching the middle of the second peduncular segment, and, terminally, obtusely pointed. The distal two peduncular segments are subequal in length and the proximal one is elongated at the distal outer corner to a sharp point. The second antenna is about as long as the body without the rostrum and the telson, and furnished with a well developed scaphocerite which almost reaches the distal end of the flagellum of the first antenna, and its blade exceeds the tip of the outer spine. The mandible is divided into two parts and furnished with a palp in two segments. At the base of the second maxilliped there is a lobe on the outer margin, and this lobe extends downwards to a thin lamella which is evidently a rudimentary mastigobranchia. The upper portion of this lobe is thick and provided with a number of small branching lobes. The lump of these lobes is probably a rudimentary podobranchia. The external maxilliped reaching the distal end of the scaphocerite is four-segmented and devoid of exopodite. And its distal segment is terminally provided with a series of spinules. The first pereiopod is stout and chelate; the tip reaches the middle of the terminal segment of the external maxilliped. The second leg, which reaches the distal end of the antennal scale, is slender and bears a small chela; the carpos is subdivided into seven segments, in which the third is the longest of all, the distal one succeeds it in length, and the remaining five segments are much shorter than any of the above

described. The following three pairs of legs are like in feature and
as long as or somewhat longer than the second leg. The terminal
segment or the dactylus is short and on the posterior margin as well
as on the propodus and the murus there is a series of spinules, which
vary in number. A rudimentary mastigobranchia is provided on each
of the external maxillipeds and the anterior three pairs of legs.

   Of the abdominal segments, the first is dorsally armed with two
spinular tubercles in pair on the anterior portion, while the posterior
half is dorsally carinated in the medial line. The succeeding three
abdominal segments are dorsally strongly carinated in the medial
line, and these carinae are more or less posteriorly pointed. In the
fifth abdominal segment the anterior half is dorsally carinated in the
medial line, while the posterior half is dorsally rather flat surfaced,
and a pair of obtuse carinae extend backwards to two acute points.
The sixth abdominal segment is dorsally rounded, but the posterior
margin is produced backwards to an acute point: besides this it is
pointed backwards on each side. The lateral plates or the pleura
are rounded in the anterior four abdominal segments, while in the
fifth and the sixth they are posteriorly pointed. The telson, which
is longer than the preceding two segments together and exceeds the
tip of the uropod, is armed with two pairs of spinules on the lateral
margins and with one or two pairs on the posterior margin.

   I examined some specimens of the nearly allied species of both
sexes in the collection of Aichi-ken ; the specimens in this collection
are all larger than the present specimens ; one of them is 52 mm.
long, and the second abdominal segment of the female is broader
than that of the male.

<div align="center">

Family **Pandalidae** BATE.

Genus PANDALUS LEACH.

17. Pandalus latirostris RATHBUN.

</div>

   Pandalus latirostris RATHBUN, 1902, p. 46, Text-fig. 20, 21,

   Loc. Ôma Bay, August 18, 1927. 45 specimens.

   The body length, measuring from the tip of the rostrum to the
end of the telson, is 119 mm. in the largest and 49 mm. in the smallest.

   In the larger specimens, these samples coinside with the description

of RATHBUN in all respects but in the armature of the rostrum. The
rostrum is armed with 14 to 18, very rarely 20, above, of which 4
or 5 are on the carapace, and with 9 to 13 teeth on the lower margin.
In all but one specimen the rostrum is armed with a subterminal
tooth on the upper margin.

The body is nearly naked and smooth, but in the large specimens,
there are some areas beset with very short hairs on the gastric region
and near the lateral margin of the carapace.

In the smaller specimens, there are some variations in relative
dimensions. The rostrum is comparatively longer, and it is one and
two-thirds times as long as the carapace; therefore, the carapace,
including the rostrum, is longer than the abdomen with the telson.
The scaphocerite of the second antenna is also elongated, correspond-
ing to the length of the rostrum, so that the outer maxilliped does
not reach to the middle of the scaphocerite. The third pair of legs
is long, comparing with the length of the second leg, and it exceeds
the distal end of the second leg on the left side by one half the
length of the penultimate segment.

The colour is brownish red and marked with longitudinal darker
streaks.

General Distribution: Muroran and Tokyo.

18. Pandalus sp.
(Pl. XVI, figs 7-12).

Loc. Ômashimote. August 18, 1927. 1 specimen, probably male.

The specimen is 20,3 mm. long, measuring from the base of the
rostrum to the end of the telson.

The body is naked and its surface is smooth. The carapace
without rostrum is about two and one-third times as long as the ab-
domen including the telson. The blunt medial carina on the dorsal
surface of the carapace is produced to the rostrum. The rostrum,
though the terminal half is unfortunately missing in the specimen,
bears ten movable spines above and four teeth below on the proximal
half. Three of the upper spines are behind the posterior orbital
margin, and in those on the inferior margin the posterior one is the
largest. On the anterior margin of the carapace there are a strong

antennal and a small branchiostegal tooth on each side besides the rostrum.

The first antenna is provided with a small stylocerite, which is terminally rounded, and bears two flagella of the length of about one and a half times the peduncle. The scaphocerite of the second antenna is somewhat shorter than the carapace excluding the rostrum. It is narrow in shape, but broader near the base, gradually tapering to the distal extremity, where the external spine projects far forwards beyond the blade. At the broadest point it is about seven times as long as broad. The flagellum is missing. The mandible is distinctly divided into two processes, the incisor and the molar, and bears a well developed palp, which consists of three segments and almost reaches the extremity of the incisor process. In the segments of this palp the proximal is narrow at the base and the distal is somewhat longer than any of the foregoing segments. The upper lobe of the first maxilla is terminally rounded. In the second maxilla, the laminar exopodite extends backwards into the branchial chamber, and is fringed with long hairs on its posterior extremity as is commonly seen in this genus. The medial lobe or the endopodite, tapering to the extremity, is distally curved inwards. In the remaining three lobes, which correspond to the protopodite, the inner margin of the basal is rather concave, while the anterior two lobes are well developed and their margins are rounded. The first maxilliped is provided with a well developed epipodite which is divided into two lobes, while in the second maxilliped it is smaller than that of the preceding pair, and a small podobranchial plume is attached at the base. The third maxilliped somewhat exceeding the distal end of the antennal scale is five-segmented, and the basal segment is furnished with a rudimentary mastigobranchia. The second segment is very short; the third, terminating in two spinules, is the longest and the basal half is excavated at the dorsal surface. It is as long as two and a half times the length of the fourth and is one and one-fifth times as long as the terminal segment, which terminates in several numbers of spinules. Each of the anterior four pairs of legs is also furnished with a rudimentary mastigobranchia. The first leg is styliform, six segmented, and a little shorter than the third maxilliped. The second pair is chelate, bears the carpos subdivided into more than 50 articles in the left, while

in the right it is divided into about 20 articles. The latter is some-
what longer than the third maxilliped, while the former is much
longer than these. The third leg is stout and a little shorter than
the second leg of the left; the merus is armed with five spinules on
the posterior margin, the carpos with a spine, and the propodos,
being a little compressed, is somewhat dilated at the posterior margin,
where a number of spinules are provided. In the fourth leg the merus
is armed with four spinules on the posterior margin and three on the
outer surface. The dactyli of the posterior three pairs of legs are
short and armed with a series of spinules on the posterior margins.

The abdomen is laterally compressed and dorsally rounded. The
sixth segment is somewhat longer than the preceding two segments
together. The telson, which is one and a half times as long as the
sixth, is dorsally armed with four pairs of spinules and terminates in
three pairs of spinules.

The species is closely allied to *Pandalus montagui tridens* RATHBUN[1]
from Alaska, but it seems to be quite different from this in two main
points, that is, in the shape of the antennal scale and in that the
sixth abdominal segment is stouter. The fact that the antennal scale
tapers to its extremity indicates that the species has some affinity to
the genus *Notocaris*.

Only a single specimen being found in the collection and the
terminal half of its rostrum being missing, it is considered better to
reserve the naming of the present species, although it is quite certain
that it belongs to *Pandalus*, and it is unable to identify it as any
species of the genus hitherto known to science.

Family Crangonidae BATE.

Genus CRANGON FABRICIUS.

19. Crangon affinis DE HAAN.

Crangon affinis DE HAAN, 1849, p. 183.
Crangon propinquus STIMPSON, 1860, p. 25; RATHBUN, 1902, p. 42, BRASHNIKOW,
          1907, p. 84.
Crangon hakodatei RATHBUN, 1902, p 42, Text-fig 15
Crangon consobrinus DE MAN, 1907, p. 405.
Crangon cassiope DE MAN, 1907, p. 466.

[1] RATHBUN, 1902, p. 901.

Japanese name: Yebizyako.
Loc. Between Yunoshima Isl. and Asamushi. 5–6 fms. Sea-weeds.
April 29, 1926. 7 males, 7 egg-bearing females and 2 im-
matures.

The coast of Namiuchi, Heinai. Sand, gravels and sea-weeds.
July 17, 1926. 1 egg-bearing female.

Moura. 5 fms. Sand and sea-weeds. July 23, 1926. 3 egg-
bearing females.

On the line between Futago-zaki and Ôshima Isl., off Urata.
24 fms. Sandy mud. July 30, 1926. 1 female with eggs.

On the line between Futago-zaki and Ôshima Isl., off Cape
Aburame-zaki. 27 fm. Sandy mud. July 30, 1926. 1 female
with eggs.

Off Futatsuya. 31 fms. Sand. July 31, 1926. 1 egg-bearing
female.

Moura. 5 fms. Sand, sea-weeds. August 16, 1926. 2 egg-bearing
females.

Off Noheji. 5 fms. Sea-weeds. August 22, 1926. 2 egg-bearing
females.

Off Yomogita. July 23, 1927. 5 egg-bearing females.

Off Tsubakiyama. July 24, 1927. 1 female with eggs.

On the line between Yokohama and Kanita, off Noheji. July
25, 1927. 1 female with eggs.

On the line between Ôshima Isl. and Aomori, off Itazaki.
August 10, 1927. 2 males, 5 egg-bearing females.

Ôma Bay. August 18, 1927. 3 males and 1 egg-bearing female.

The entrance of Fukura Bay. August 19, 1927. 3 young
specimens.

Examining numerous specimens, I noticed that the species is quite
variable and many intermediate forms between the so-called species
C. propinquus, C. hakodatei, C. consobrinus and *C. cassiope* are found.
I therefore agree with Prof. H. BLASS (1914), the species stated above
are nothing but synonyms of *C. affinis* DE HAAN.

General Distribution: Japan and Corea.

Family **Palaemonidae** BATE.

Genus LEANDER DESMAREST.

20. **Leander serrifer** STIMPSON.

Leander serrifer STIMPSON, 1860, p. 41; ORTMANN, 1891, p. 525, Pl. 37, Fig. 17.

Japanese name : Sujiyebimodoki.

Loc. Off Fukikoshi. 22 fms. Sand and gravels. August 23, 1926.
    5 egg-bearing females.
        Sai Bay. August 17, 1927. 1 female with eggs.
    The rostrum is armed with 11 or 12 teeth above, of which 3 or
4 are on the carapace ; and 3 or 4 teeth are on the inferior margin
of the rostrum.

General Distribution : Hongkong ; Amoy ; Loochoo Isls. ; Japan :
Tokyo Bay, Tanagawa.

Tribe THALASSINIDEA.

Family **Callianassidae** BATE.

Genus CALLIANASSA LEACH.

21. **Callianassa subterranea** (MONTAGU)
        var. **japonica** ORTMANN.

Callianassa petalura STIMPSON, 1860, p. 23.
Callianassa subterranea japonica ORTMANN, 1892, p. 56, Pl. 1, Fig. 10 a, DOFLEIN, 1902, p. 644; BALSS, 1914, p. 91.

Japanese name : Sunamoguri.

Loc. The mouth of the Tanabe River. Mud. August 11, 1926. 2
    males and 3 females.
        Moura. 5 fms. Sand and sea-weeds. August 26, 1926. 1 female
    with eggs.

General Distribution : Japan : Tokyo Bay, Simoda, Bingo, Ha-
    kodate.

Genus GEBIA LEACH.

22. **Gebia major** DE HAAN.
            (Text-fig. 4).

Gebia major DE HAAN, 1849, p. 165, Pl. 35, Fig. 7; MIERS, 1879, p. 52; ORTMANN,
        1892, p. 54, Pl. 1, Fig. 7.
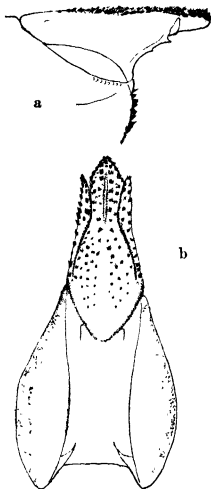
Text-fig 4. *Gebia major* DE HAAN, fully
grown specimen
  a Anterior half of carapace (×2).
  b Carapace, dorsal aspect (×2)

Japanese name: Anazyako.

Loc. Asadokoro. July 13, 1927. 3 males and 2 females.

General Distribution: Japan: Katsura, Koda Bay, Tokyo Bay, Sagami Bay.

23. **Gebia affinis** n. sp.
(Text-fig. 5).

Loc. Asadokoro. July 6, 1926. 2 males.
Nonai. August 15, 1927. 1 young female.

Of the specimens from Asadokoro, one is 42,8 mm. and another 33,5 mm. long from the tip of the rostrum to the end of the telson. I was able to examine one female specimen of this species, which is 45,8 mm. long, collected by Mr. HIROAKI AIKAWA from the coast of Haneda near Tokyo.

The species very closely resembles *G. issaeffi* BALSS from Vladivostok and *Gebia major* DE HAAN, but it is distinctly different from these.

The frontal margin of the carapace is similar to that of *G. major*, consisting of three anteriorly directed processes, the medial one being larger and longer than that on each side. In the present species, not only is the medial process somewhat narrower and more acutely pointed, but the lateral processes are more apart from the
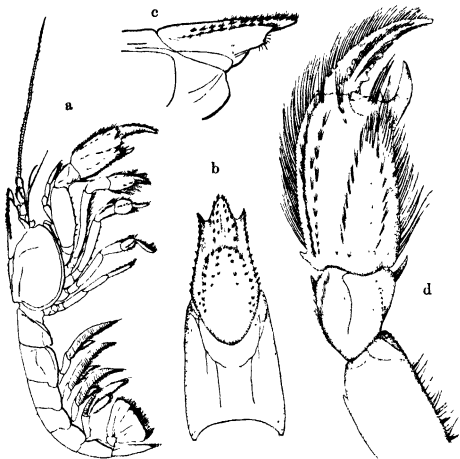
Text-fig. 5   *Gebia affinis*, n. sp.

a. Entire animal, view from right side.   *b* Carapace, dorsal aspect (×4)
c. Anterior half of carapace, view from right side. (×4)   *d* Terminal half
of 1st pereiopod, view from out side. (×6).

medial in the dorsal aspect than those of *G. major*. Viewing it from
the lateral side, the medial process shows in profile a line continuous
with the upper margin of the carapace, and the lower margin obliquely
ascends to acuminate to the tip; while in *G. major* the line of the
upper margin is continuous with that of the carapace at first, but
descends abruptly downwards at the tip and the lower margin shows

a nearly horizontal line in the smaller specimens. In the full grown
specimens, the lower margin is slightly ascended to the extremity,
where, however, it is not so acutely pointed as that of the present
species.

The pereiopod is alike in both sexes and similar in shape to that
of *G. issaeffi*. The meros is stouter than that of *G. major* in the
specimens of similar size, about two and a third times as wide as
long, and its outer surface is smooth and devoid of hairs; while the
teeth on the lower margin are sharply pointed. The carpos is armed
with a series of small acutely pointed teeth near the upper margin;
this series of teeth is more prominent than that found in *G. issaeffi*
and the terminal tooth is much stronger than any of the rest. The
carpos is armed with two strong teeth on the distal margin, one on
the lower margin and stronger than the other which is on the upper
margin. The palm of the chela is about twice as wide as long and
its surface is nearly smooth and superiorly provided with three obtuse
longitudinal carinae which are fringed with series of long hairs. In
these carinae the medial one is the most prominent and is guarded
with a strong tooth on each of the proximal and the distal ends of
the carina. Between this and the outer carina it is longitudinally
furrowed. The palma is also provided with two rows of hair bundles
on the outer surface; and these two rows meet behind the hinge of
the finger, where hairs are scattered in numerous bundles. The lower
margin is slightly carinated and furnished with long hairs. The anterior
prolongation of the propodos or the polex is armed with a strong
triangular tooth at the base of the inner margin. The dactylos or
the movable finger is provided with three rows of tubercles, one on
each of the upper and lower margins, and another on the outer sur-
face. In the latter the tubercles are prominent, while in the former,
except the proximal one or two, they are mostly much less prominent.
Beside these, on the upper margin and on each side of the lower
margin, hairs are studded in a series, and another series of hairs is
just below the said tubercular series on the outer surface. The hairs
on the upper margin are prominent and are longest at the base
diminishing terminally in length. In other respects the species coin-
cides with *G. major*.

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(STIMPSON, W. 1864. Proc. Acad. Nat. Sc. Philadelphia.)

EXPLANATION OF PLATE.

Figs 1–6. *Paraspirontocaris kishinouyei*, n gen. n sp.

    Fig. 1. Entire animal, view from left side (×5).

          2.   ,,   ,,   , dorsal aspect (×5).

          3. Mandible.

          4. 2nd maxilla.

          5. 1st maxilliped.

          6. 2nd maxilliped.

Figs 7–12. *Pandalus* sp

    Fig. 7. Entire animal, view from left side. (×5).

          8. Mandible.

          9. 1st maxilla.

        10. 2nd maxilla.

        11. 2nd antenna of right side, dorsal aspect. (×10).

        12. 3rd maxilliped (×10)

Note on the Physico-chemical Conditions of the Habitat of Nereis japonica IzuKA.[1]

By

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In the last few years, I had opportunities to use *Nereis japonica* for various purposes, during my sojourn at the Marine Biological Laboratory of Asamushi. Some of the results of my experiments already appeared in a preliminary form (NOMURA 1926), and the rest will also appear in this journal in the near future. This animal is becoming more and more the favorite material for the workers at the laboratory, and it will be of use to put on record some of my observations and brief experiments concerning the life conditions of the worm.

The geographical distribution of the animal covers a wide range extending from the Inland Sea (Seto Uchi) to Sakhalin, and both on the coasts of the Sea of Japan and the Pacific (IzuKA, 1912). The animal may well be said eurythermal. The species under consideration has been recorded from various localities, and is believed to live in brackish waters, as do many allied forms.

Brackish water animals in general are adapted to variations of wide ranges in physical and chemical conditions of the habitat. The characteristics of the brackish water are not only low concentration but also more or less fluctuation in its salt content (LENZ, p. 201), and the range of fluctuation may depend on various circumstances of the locality. As to the salinity or its variations in the habitat of *Nereis japonica*, however, no concrete and exact data are at hand, as far as I am aware.

During my experiment, some one pointed out to me that *Nereis japonica* lives in brackish water and it would be more preferable to work with brackish water than with sea water. I tried to ascertain

this point, therefore, and made a few observations and brief experi-
ments. But the data presented here are not complete as bearing
upon physical ecology of the animal, and are rather, intended to
urge further studies by are workers at our laboratory, who are in-
terested in this line.

It is a well known fact that many species of *Nereis* are euryhaline,
and moreover, they are the most resistant, among their associates in
brackish water, against the changes in salinity. Examples of this fact
are very common.

FERRONNIÈRE reported that, at the mouth of the Loire, in going
upstream, representative marine animals and plants dropped out in
the following order · — *Arenicola marina, Lineus gesserensis, Carcinus
moenas, Sphaeroma serratus, Fucus* and *Lichina, Balanus, Amphitrite,
Cardium edule, Heterochaeta, Vermiculatus, Phargmites communis,
Boccardia ligerica, Nereis diversicolor, Palaemon edwardsi.* From this
fact, it is evident that *Nereis* is one of the most resistant animals to
fresh water. *Nereis japonica* is closely allied to *Nereis diversicolor*
and might easily be confounded with the latter. Indeed, it was once
identified with *N. diversicolor* by MARENZELLER, and was later
separated from the latter by IZUKA (1908, p. 166; see also FAGE et
LEGENDRE, p. 118).

From the foregoing, we may also expect an extremely wide range
of variation in the physical conditions of the habitat of *Nereis japonica.*
To ascertain this point, the auther made a few determinations of the
properties of the water of the habitat of the animals, which he used
in his experiments.

The supernatant water over the sandy bottom of the habitat near
the mouth of the small river, Shiodachi Kawa, at Asadokoro, was
collected in a hard glass flask of one liter capacity, tightly stoppered,
and brought to the laboratory, where examinations were made.

The hydrogen ion concentration of the medium is well recognised
now-a-days as one of the most important factors determining the
distribution of many organisms. Beside specific gravity and chlorine
content, therefore, the approximate pH value of the water was
colorimetrically determined. The results are given in the accompanying
table and show clearly that the salinity of the water of the habitat
varies over a considerably wide range from the full salinity of the

TABLE I.

Sample of water	Specific Gravity		Chlorine content gm per L	pH
	Observed S_t^t	Corrected S_t^{15}		
Water of Habitat.				
Oct 17	$S_4^{17} = 1.0176$	1.01729	13.55	8.1
Oct. 26	$S_4^{14.5} = 1.02439$	1 02430	18.36	8.3
Dec 6	$S_6^{10} = 1.0021$	—	1.75	7 5
Sea Water (Laboratory)				
Oct 21	$S_t^{15} = 1.0245$	1.02.5	18.30	8 6
Dec 6				8.6
Fresh water, (Laboratory)				
Oct 21	$S = 1.000$	1.000	0 027	8.9
Dec 6				8.8
Distil water		—	—	6.6
Mixture				
{F.w 3 parts} {S.w 7 parts}			12 82 (calculated)	8.8
{D w 3 parts} {S w 7 parts}			12.81 (calculated)	8.6

Abbreviations F W = Fresh water, S w = Sea water, D w. = Distilled water

ordinary sea water[1] nearly to that of oligohaline[2] water. The animal, therefore, must be able to resist a very low salinity. The changes in salinity of the water are, of course, accompanied by changes of various other factors, such as osmotic pressure and hydrogen ion concentration, which may in turn affect the physiological processes of the animal. For the experimental work, therefore, we must always use as the medium the sea water of approximately the same salinity. Bearing this in mind, the following brief experiments were carried out to compare the effects of brackish and sea water.

The glass vessel containing the animals, with a layer, one or two inches deep, of sand from their habitat, was circulated continually

[1] The specific gravity of the sea water in the vicinity of the Asamushi Laboratory ranges from 1.022 to 1.024 in the spring, and from 1.023 to 1.025 in the winter (YAZAKI, pp. 287–8)　　[2] LENZ, p. 201.

with sea water from the tap in the laboratory, or the sea water in
the vessel was renewed from time to time, and the animals were
found to survive in a good condition for weeks or months. When,
however, tap water or distilled water was added gradually to the sea
water in the container, so that the salinity decreased to about 70 per
cent. of that of the ordinary sea water, as was the case with the
water sample of October 17 (table 1), the animals became sluggish
and inactive as if narcotised, and could not survive so long as in
ordinary sea water. *Nereis japonica*, therefore, at least that of the
locality above mentioned, is better adapted to the sea water of ordinary
salinity than to mesohaline or oligohaline[1] waters.

     Nereis in general lives in the sand or mud of the sea shore, between
the tide marks. When the tide is low, fresh water from the river or
rain water from the land would dominate over the habitat of the animal.
But the sea water may be retained in considerable amount between
the particles of sand, and the salinity at a small depth in the bottom
may not be thoroughly altered, as might be supposed at the first
glance, before the high tide comes back. *Nereis japonica* makes a
temporary tube of sand particles cemented with mucus secreted round
the body, though it is neither perfect nor elaborate. Mucus is known,
however, as a very effective means for protection against osmotic
changes in the medium (PAUL BERT). These circumstances seem to
aid the animal in surviving a wide range of external changes.

     In the experiments with dilute sea water, above mentioned, it was
tried to imitate some of the natural conditions, by making a layer of
sand from the habitat, in the bottom of the vessel containing the
animals. The small scale of the arrangement, however, seems not to
have allowed the conditions in the vessel to reproduce natural condi-
tions to sufficient extent.

     The cause of death of the animals in diluted sea water can not
be attributed to high pH values of the water, as the animals died
both in the mixtures of fresh water—sea water and distilled water—sea
water, and showed no remarkable difference between them, while the
pH value of the latter is equal to that of the laboratorry sea water,
in which the animals survived quite a long time in good condition.

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[1] LENZ, *loc. cit.*

Osmotic relations must be mainly responsible.

For the experimental work with *Nereis japonica*, therefore, at least with animals from the locality mentioned above, ordinary sea water is preferable as medium to dilute sea water. And in all my experiments mentioned at the beginning of this paper, the ordinary sea water was exclusively used.

Thanks are due to Mr. NONAKA, assistant of the laboratory at the time I worked there, for his kindness in determining the chlorine content of water samples.

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The Vegetation of Mt. Hakkôda.[1]

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(With Plates XVII–XX, and 4 Text-figures).

INTRODUCTION.

Mt. Hakkóda stands on the northeastern end of Honshiu, the main island of Japan, shelving northwards its gentle slope as far as Mutsu Bay, 140°53′ L. E. and 40°40′ L. N. in the geographical position. In reality it is an extinct volcano group, consisting of nine main peaks. The highest of these is the Ôdake rising 1585 meters above sea-level, and is formed of the pyroxene-andesites and their agglomerates.[2]

Ecologically, it is indeed a place of utmost interest, because it presents many different kinds of vegetation for a rather limited area owing to the varied edaphic and climatic conditions to be found there.[3] The following are only a few instances of this: —

1. A large number of the alpine plants, shrubs as well as herbs, are abundant on the peaks of Ôdake, Idodake, Akakuradake, Takada-ôdake, etc.

2. Deciduous and coniferous forests cover the most part of these peaks.

3. Two large craters exist; one is at Ôdake, another at Idodake. A crater-lake is at Akakuradake.

4. Although the mountain is entirely in a dormant stage, there are still found sulphuretted hot springs in the surrounding region, being especially numerous in the neighbouring districts of Sukayu, — an indication that the volcano has but recently ceased to be active.

Fig 1. View of Mt Hakkoda (S side) seen from Peak Komagamine showing *Abies Mariesii*-forest in the spring (after a phot).

Fig 2. View of Mt Hakkoda (N E side) seen from Peak Takadaodake in the spring (after a photograph).

The largest of them is the Digokunuma in the Botanical Garden which probably erupted most recently and even now jets actively the sulphuretted hydrogen There are no historical records regarding the eruptions of this mountain, but we can observe the evidence of past volcanic activity upon the vegetation of this mountain

5   There are numerous streams which originate from forests and snow breaks   The most prominent is the Arakawa   As it is young it forms a V shaped valley with some water falls during its course having its effect on special plant formations   Cold spring are not rare   one of them is situated in Kaminota, 3~4℃, in mid summer

6   Marshes and ponds are able to be found here and there   Suirenmuma and Kenasitai are two examples of them

7   Bare regions, rocky as well as detritus, consisting of sulphate and aluminium compounds of volcanic origin, are also found

8   Various kinds of moor or boggy places are scattered about

9   Different stages of successions of various sorts, are also to be observed most easily here and there

10   Large masses of snow which usually remain till the end of July are to be seen in certain places

There was no very satisfactory study of the vegetation of the mountain before the establishment of the Botanical Laboratory, but of what existed, that by TATEWAKI [1] in 1927, was most important The main object of this paper is the general survey of the vegetation within the following principal area   The region includes   The Ara kawa (700 m) — Sinyu — Sukayu — Kenasitai — Akakuradake — Idodake — Ōdake — Isikuradake — Suirennuma — the Arakawa (950 m)   The data presented in this study are based on my own observations and researches during the last four years [2]

I wish to express here my sincere thanks to Prof Dr Y YOSHII to whose guidance I am indebted for this work

THE PLANT-COMMUNITIES.

The plant-communities are divided here into the seven main forma-tions : —

I. Forest Formations.   II. Aquatic Formations.
III. Moor Formations.   IV. Herbaceous Land-Formations.
V. Shrub Formations.   VI. Dwarf-shrub and Dwarf-herb
                                   Formations.

I. Forest Formations.

It is a striking fact that the floral composition of the forest is very simple, mainly consisting of *Fagus Sieboldi, Abies Maresii*, mixed with a few of *Quercus crispula, Betula Ermani* with *Sasa kurilensis*. There is a complete absence of *Picea* which is widely distributed in middle Honshiu and Hokkaido.

1. *Pterocarya rhoifolia-Alnus hirsuta*, v. *sibirica*-Association.

This association is well developed along both sides of the Arakawa. The dominant species are *Pterocarya rhoifolia* and *Alnus hirsuta*, v. *sibirica*. The trees of *Quercus crispula, Carpinus cordata* and *Acer pictum*, ssp. *eupictum*, *Acer japonicum*, v. *typicum*, *Acer tenuifolium*, *Ulmus laciniata* are scattered here and there. Along the more ex-posed course and near the water, the shrubs of *Alnus pendula, Dier-villa japonica, Salix sachalinensis* and *Salix Bakko* have occupied the space. The following list is an enumeration of plants which commonly are plentifully found as the undergrowth : — *Rodgersia podophylla, Petasites japonicus, Angelica polyclada, Angelica ursina, Conioselinum univittatum, Aruncus vulgaris, Glaucidium palmatum, Thalictrum tuberiferum, Viola brevistipulata, Platanthera decipiens, Saussurea Tanakae, Saxifraga fusca, Oxalis Acetosella*, v. *japonica, Adenophora remotiflora, Matteuccia orientalis, Serratula deltoides, Tiarella polyphylla, Cardiocrinum Glehni, Mimulus sessilifolius, Al-lium Victoralis, Pleurospermum kamtschaticum, Veratrum nigrum, Adiantum pedatum, Polygonum sachalinense, Trillium apetalon, Viola vaginata, Oenanthe aristata*, v. *montana, Aralia cordata, Glaucidium palmatum, Ranunculus hakkodensis, Arisaema amurense, Saxifraga*

cortusaefolia, v. *typica*, *Diphylleia Grayi*, *Dryopteris viridescens*, *Dryopteris africana*. *Anemone altaica*, *Galium triflorsforme*, *Galium kamtschaticum*, v. *oreganum*, *Elatostema involucrata*, *Elatostema laetevirens*.

Some species, e. g., *Aconitium pallidum*, *Anemone flaccida*, *Gymnadenia Kinoshitai* were observed occasionally, forming each a small community of its own.

On the rocks and the rocky cliffs along the water the following small plants are common: — *Patrinia gibbosa*, *Lilium medeoloides*, *Calamagrostis sachalinensis*, *Potentilla Dickensii*, *Artemisia Keiskeana*, *Saxifraga cortusaefolia*, v. *typica*, *Blechnum amabile*, *Woodsia polystichoides*, *Thalictrum minus*, v. *nanum*, *Hypericum kamtschaticum* and *Lactuca dentata*, v. *Thunbergii*.

Communities of *Polygonum sachalinense*, *Fragaria Jinumae*, *Rubus spectabilis*, ssp. *vernus*, *Artemisia vulgaris*, v. *integrifolia* were noticed covering the exposed and extensive area where a landslide had recently occurred. Probably they show the first stage of succession to this association.

2. *Fagus Sieboldi*-Association

This Fagetum extends widely over the mountain as far as to the elevation of about 900 m. forming a pure stand. The following trees and shrubs are sometimes found among them in small numbers: — *Magnolia hypoleuca*, *Cornus controversa*, *Fraxinus longicuspis*, *Aesculus turbinata*, *Micromeles alnifolia*, *Phellodendron amurense*, *Prunus Grayana*, *Acer tenuifolium*, *Magnolia salicifolia*, *Viburnum furcatum*, *Kalopanax sciadophylloides*, *Sorbus Aucuparia*, *Lindera umbellata*, *Aucuba japonica*, v. *borealis*, *Daphniphyllum humile*, *Skimmia japonica*, *Ilex crenata*, v. *typica*, f. *genuinea*, *Ilex leucoclada*, *Daphne kiusiana*, *Evonymus striatus*, *Celastrus articulatus*, *Actinidia Kolomicta*, *Rhododendron Albrechtii*, *Cephalotaxus drupacea*, *Lonicera Morrowii*, *Hydrangea opuloides*, v. *acuminata*, *Hydrangea petiolaris*, v. *cordifolia*, *Schizophragma hydrangeoides*, *Vitis Coignetiae*, etc.

The herbaceous undergrowths are as follows: — *Maianthemum bifolium*, *Crawfurdia trinervis*, *Polygonatum falcatum*, *Panax Schinseng*, v. *japonicum*, *Diphylleia Grayi*, *Polystichum tripteron*, *Oreorchis patens*,

f. *gracilis*, *Orchis aristata*, *Pirola media*, *Oxalis Acetocella*, v. *japonica*, *Trillium apetalon*, *Glaucidium palmatum*, *Disporum sessile*, *Streptopus amplexifolius*, *Clintonia udensis*, *Astilbe congesta*, *Streptopus ajanensis*, v. *japonica*, *Viola vaginata*, *Smilax Oldhami*, *Eupatorium sachalinense*, *Paris tetraphylla*, *Smilacina japonica*, *Gentiana Makinoi*.

3. *Fagus Sieboldi-Abies Mariesii*-Association.

This association occurs just between the associations of *Fagus Sieboldi* lower and *Abies Mariesii* upper, viz., on the average 900~1100 m. above sea-level *Sasa kurilensis* mostly predominates as the undergrowth. In some places, e. g., Digokunuma and Isikuradake, etc., *Betula Ermani* was found codominant. It seems to the writer that this association represents rather an intermediate stage destined to be suppressed at last by the *Fagus Sieboldi* or *Abies Mariesii*-association. Mixed shrubs are as follows: — *Vaccinium hirtum*, v. *Smalli*, *Vaccinium axillare*, *Tripetaleia paniculata*, *Rhododendron Albrechtii*, *Menziesia pentandra*, *Lindera umbellata*, *Ilex leucoclada*, *Daphniphyllum humile*, *Ilex Sugeroki*, ssp. *brevipedunculata*, *Skimmia japonica*, *Rhus Toxicodendron*, v *vulgaris*, f. *radicans*, *Ilex crenata*, v. *typica*, f. *genunea*, *Epigaea asiatica*, *Magnolia salicifolia*, *Kalopanax sciadophylloides*, *Hydrangea petiolaris*, v. *cordifolia*, *Schizophragma hydrangeoides*.

As the undergrowth the following herbs are commonly present : — *Asarum Sieboldii*, *Streptopus amplexifolius*, *Diphylleia Grayi*, *Panax Schinseng*, v. *japonicum*, *Trillium apetalon*, *Paris tetraphylla*, *Clintonia udensis*, *Platanthera ophrydioides*, *Crawfurdia trinervis*, *Cornus canadensis*, *Maianthemum bifolium*, *Trientalis europaea*, *Peracarpa carnosa*, *Oxalis Acetosella*, v. *japonica*, *Viola brevistipulata*, *Myrmechis japonica*, *Streptopus ajanensis*, v. *japonica*, *Glaucidium palmatum*, *Astilbe congesta*, *Lycopodium serratum*, *Plagiogyria Matsumuraeana*, *Blechnum nipponicum*, *Blechnum castaneum*.

4. *Abies Mariesii*-Association.

It is a striking feature that *Abies Mariesii*-forest develops extensively. This is widely spread on the average from 1150 m. to 1450 m. above sea-level and almost completely surrounds the mountain. The

western side of Idodake is one of the most luxuriant and pure stands in the mountain. As one ascends the mountain sides, the tree gradually becomes smaller and smaller until it is entirely replaced by *Pinus pumila*. The upper limit of this association is rather irregular, owing mainly to the climate and topography. Additional trees and shrubs are *Acer Tschonoskii*, *Acer spicatum*, v. *ukurunduense*, *Acer japonicum*, v. *typicum*, *Viburnum furcatum*, *Rhododendron brachycarpum*, *Rhododendron Albrechtii*, *Corylus rostrata*, v. *Sieboldiana*, *Prunus Grayana*, *Prunus nipponica*, *Sorbus Aucuparia*, *Ilex Sugeroki*, ssp. *brevipedunculata*, *Schizophragma hydrangeoides*, *Actinidia Kolomicta*, *Hydrangea paniculata*, *Leucothoe Grayana*, v. *Maximowicziana*, *Vaccinium hirtum*, v. *Smallii*, *Tripetaleia paniculata*, *Tripetaleia bracteata*, *Menziesia pentandra*, *Salix Reinii*, *Ilex leucoclada*, *Ilex crenata*, v. *typica*, f. *genuinea*, *Echinopanax horridus*, *Rubus vernus*, *Ilex rugosa*, *Ilex rugosa*, v. *Fauriei*, *Epigaea asiatica*. The undergrowth is mostly characterised by the predominance of *Sasa kurilensis*. The following species are found in this association: — *Clintonia udensis*, *Plagiogyria Matsumuraeana*, *Diphyllea Grayi*, *Cacalia auriculata*, v. *kamtschatica*, *Crawfurdia trinervis*, *Trientalis europaea*, *Mitchella repens*, v. *undulata*, *Cornus canadensis*, *Viola Selkirkii*, *Peracarpa carnosa*, *Maianthemum bifolium*, *Coptis trifolia*, *Paris tetraphylla*, *Trillium apetalon*, *Oxalis Acetosella*, v. *japonica*, *Lycopodium serratum*, *Chimaphila japonica*, *Pirola japonica*, *Listera nipponica*, *Ephippianthus Schmidtii*, *Monotropa uniflora*, *Goodyera repens*, *Tiarella polyphylla*, *Aruncus vulgaris*, *Athyrium melanolepis*, *Dryopteris Christiana*, *Dryopteris polylepis*, *Dryopteris dilatata*, v. *deltoidea*, *Heloniopsis brevicapa*, *Carex Wrightii*, *Poa alpina*, *Lactuca dentata*, v. *albiflora*, *Shortia soldanelloides*, v. *genuina*, f. *typica*, *Anemone gracilis*, *Streptopus ajanensis*, v. *japonica*, *Cnidium ajanense*.

II. Aquatic Formations.

1. *Nymphaea tetragona*, v. *angusta*, sv *orientalis*-Association.

This is a noted feature of the several ponds of Suirennuma, bearing numerous white flowers in mid-summer. Often it is accompanied by *Potamogeton Franchetii*, *Eleocharis palustris*, *Phragmites communis*.

In the marginal strand there occur often *Menyanthes trifoliata, Lobelia sessilifolia*.

The principal ecological demands for this association are a moderate depth of water (ca 1.5 m) and a muddy substratum.

2. *Menyanthes trifoliata*-Association.

This community occurs here and there. It represents often the transitional stage from the aquatic to the dried association. We have observed that the Nymphaeetum is replaced by this association at the decrease of the depth of water, and this Menyanthetum is replaced by *Scheuchzeria palustris* when the ground becomes dry. In other words the succession runs in the following manner : — Nymphaeetum → Menyanthetum → Scheuchzerietum → Grass Land-Formation.

3. *Phragmites communis*-Association.

The association was observed in the swamps at Digokunumayati, Akamizuzawa etc. Its floral composition varies according to the locality. *Ligularia sibirica, Lobelia sessilifolia, Inula cliliaris, Sparganium simplex* and *Typha latifolia* are the most common companions.

4. *Saxifraga japonica*-Association.

Hydrurus grows in the clean and cold water of the Arakawa, attached to the rock-bed in mid-summer. A small community of *Saxifraga japonica* with a white flower is often developed at the place where spring water flows into the main stream.

III. Moor Formations.

The *Sphagnum* bog is developed rather extensively in this locality on the exposed places, e. g., Kenasitai, Suirennuma, Akamizuzawa, Digokuyati etc. Generally speaking the developmental age is not so long, for the substratum bearing the organic matter is not very thick. It is a characteristic that *Drosera rotundifolia, Oxycoccus palustris,* v. *intermedium* and *Ledum palustre,* v. *nipponicum* usually occur, being

accompanied by some subordinate elements. For an example, at Suirennuma we found the following plants in addition to the above-mentioned principal elements : — *Andromeda polifolia, Carex stellulata, Carex stellulata,* v. *Omiana, Carex Michauxiana, Carex aphyllopus, Eiophorum gracile, Rhynchospora Yasudana, Rhynchospora alba, Scirpus cyperinus,* v. *Eriophorum, Juncus prismatocarpus,* v. *Leschenaultii,* sv. *pluritubulosus, Juncus effusus,* v. *decipiens, Luzula campestris,* v. *pauciflora, Aletris foliata, Shortia soldanelloides,* v. *genuina,* f. *typica, Coptis trifolia, Narthecium asiaticum, Lilium Maximowiczii, Hosta japonica,* v. *angustifolia, Sanguisorba tenuifolia,* v. *alba, Viola vere-cunda,* v. *typica, Ligularia calthaefolia, Inula ciliaris, Pogonia minor, Solidago Virgaurea.*

IV. Herbaceous Land-Formations.

The formations include various types of associations. They usually develop arround the ponds and on the alluvial soils along the water courses. Some examples of them are as follows : —

1. *Lysichiton camtschatense*-Association.
2. *Veratrum stamineum*-Association.
3. *Trautvetteria palmata,* v. *japonica*-Association.

V. Shrub Formations.

1. *Alnus-Sorbus-Betula*-Association.

The association of this type is rather poorly developed. Two examples from Ôdake are given here; one is at Hasigonobori (1250–1450 m), the other at Sakuranuma (1360 m). These are so densely covered by shrubby thickets that they are almost impenetrable, dominated by *Alnus Maximowiczii, Sorbus Aucuparia* and *Betula Ermani.* The elements of this association are, by nature, greatly affected by their environment. In the case of Hasigonobori, *Acer Tschonoskii, Acer spicatum,* v. *ukurunduense, Salix Reinii, Viburnum furcatum, Prunus nipponica, Prunus kurilensis, Acer japonicum,* v. *typicum, Taxus cuspidata, Tsuga diversifolia* are found growing mixed with the above-mentioned dominant species. The following list is an enumeration of its undergrowth : — As shrubs, *Spiraea betulifolia, Echinopanax*

horridus, *Rubus vernus*, *Hydrangea paniculata*, v. *floribunda*, *Rhodo-
dendron Albrechtii*. As herbs, *Cacalia auriculata*, v. *kamtschatica*,
Diphylleia Grayi, *Tiarella polyphylla*, *Clinopodium umbrosum*, v.
japonicum, *Peracarpa carnosa*, *Maianthemum bifolium*, *Veratrum
stamineum*, *Streptopus amplexifolius*, *Streptopus ajanensis*, v. *japonica*,
Arisaema amurense, *Platanthera Takedai*, *Cnidium ajanense*, *Trillium
apetalon*, *Cornus canadensis*, *Viola Selkirkii*, *Viola brevistipulata*,
Heloniopsis breviscapa, *Aruncus vulgaris*, *Hosta japonica*, v. *angusti-
folia*, *Anemone gracilis*, *Luzula plumosa*, *Carex blepharicarpa*, *Carex
Mertensii*, v. *urostachys*, *Plagiogyria Matsumuraeana*, *Athyrium mela-
nolepis*, *Athyrium deltoidofrons*, *Dryopteris dilatata*, v. *deltoidea*,
Dryopteris polylepis, *Polystichum aculeatum*, v. *retroso-paleaceum*.

2. *Alnus Maximowiczii*-Association.

This shrubby association is observed here and there, commonly
over 1400 m. above sea-level, where a pure stand is actually
found. The companion plants are usually *Cornus canadensis*, *Vac-
cinium Vitis-Idaea*, *Orchis aristata*, *Maianthemum bifolium*, *Shortia
soldanelloides*, v. *genuina*, f. *typica*, *Aletris foliata*, *Coptis trifolia*,
Trientalis europaea, *Solidago Virgaurea*, *Pedicularis japonica*, *Carum
holopetalum*, *Paucedanum multivittatum*

3. *Pinus pumila*-Association.

This association is especially remarkable above the tree limit of
Ôdake, Idodake, Akakuradake, etc., often forming a pure impenetrable
thicket. The following plants are nearly always found as its under-
growth : — *Ilex rugosa*, *Maianthemum bifolium*, *Echinopanax horridus*,
Trientalis europaea, *Coptis trifolia*, *Cornus canadensis*, *Anemone
gracilis*, *Viola Selkirkii*, *Solidago Virgaurea*, *Lycopodium complanatum*,
Lycopodium annotinum, v. *angustatum*, *Lycopodium sitchense*, v. *niko-
ense*.

As an individual *Pinus pumila* comes down as far as 900–930 m.
above sea-level, for instance, at Digokunuma, Akamizuzawa and Sui-
rennuma. It generally creeps but sometimes stands somewhat erect
at Kenasitai.

VI. Dwarf-shrub and Dwarf-herb Formations.

1. Alpine Dwarf-shrub community.

This community is usually developed directly above the *Pinus pumila*-association. The area above 1460 m. is often covered with very compact and elastic thickets, consisting of cushion which extends rather widely and represents one of the characteristics of this mountain. The community are consisted of the following elements: — *Empetrum nigrum, Loiseleuria procumbens, Andromeda nana, Vaccinium Vitis-Idaea, Ledum palustre*, v. *yesoense, Phyllodoce aleutica, Diapensia lapponica*, v. *asiatica, Shortia soldanelloides*, v. *genuina*, f. *typica*.

2. Alpine Rocky-fields plant community.

The community of this type is developed especially on the exposed lava and volcanic bomb above 1500 m. on Idodake and Ôdake. In such places of high elevation strong winds frequently blow. And this community is a peculiar group of plants, which keep their lives well by the best adaptation to such extreme varying conditions, and usually do not make so large a community. The following plants are common to this formation: — *Pentstemon frutescens, Campanula lasiocarpa, Potentilla Matsumurae, Diapensia lapponica*, v. *asiatica, Cassiope lycopoides, Shortia soldanelloides*, v. *genuina*, f. *typica, Hypericum kamtschaticum, Pedicularis japonica, Aletris foliata, Sedum Rhodiola*, v. *congesta, Epilobium cephalostigma, Polygonum Weyrichii, Carex Doenitzii, Carex Wrightii, Carex stenantha, Deschampsia flexuosa*.

Further we have noticed that the elements of these alpine plants were distributed downwards as the chasmophytes so far as Isikuradake (1150 m). In this spot the following were observed: — *Cassiope lycopoides, Diapensia lapponica*, v. *asiatica, Andromeda nana, Vaccinium Vitis-Idaea, Potentilla Dickensii, Coptis trifolia, Carex Doenitzii*.

3. Alpine Herbaceous community.

This community develops well on the inner slopes of the craters of Ôdake and Idodake. The location is comparatively free from very strong winds and presents a rather mesophytic condition. But

generally speaking, with endless varieties in local conditions, it breaks
into very small communities, and succession can be seen here and
there. The following plants are commonly found: — *Aquilegia aki-
tensis, Arnica unlaschensis, Sedum Rhodiola,* v. *congesta, Pedicularis
japonica, Orchis aristata, Polygonum Weyrichii,* etc. *Geum pentapeta-
lum*-association of the western slope of Ôdake-crater and *Trollius
patulus*-association of the southern slope of Idodake-crater are the
most distinct and are pretty wide in their area.

Under hygrophytic conditions *Gentiana nipponica, Tofieldia nutans*
and *Pinguicula vulgaris* are frequent. *Primula nipponica-Fauria
Crista-galli*-associations are most abundant on the marginal hygrophytic
substrata of Akakuranuma, etc.

THE ALTITUDINAL ZONATION.

1. Forest region and forest limit.

It is general that the forest region is divided into the deciduous
broad-leaved tree zone (montane region) and the evergreen conifer
zone (subalpine region), based on the plant composition and elevation.
It is a regrettable matter that the environs of Sukayu, as well as the
lower region, have been damaged formerly by cuttings and fires.
On this account the boundary of the two above-mentioned zones is
not distinct and represents here a very irregular and mixed forest of
the deciduous and coniferous trees. Therefore, it is unreasonable to
decide the boundary by using an upper limit of *Fagus Sieboldi*
only, on this mountatin as is done in the European Alps by the
upper limit of *Fagus silvatica*. So, here I used a most conspicuous
conifer *Abies Mariesii* as a standard-plant, and its lower limit as the
boundary of both the deciduous and coniferous zones. This not only
eliminates the complicated phase of the mixed forest which flourishes
on the area running through Kenasitai — Sukayu — Suirennuma, but
also coincides with the lower limit of *Betula Ermani* on the whole.
In this connection, it was observed by the writer that *Abies Mariesii*
appears in association with *Fagus Sieboldi* above ca 800 m. above
sea-level in this mountain. The forest limit is more distinct. With
the increase of elevation *Abies Mariesii* becomes a more dense, and

TABLE   1.

Altitudinal distribution of the more frequent plant-communities.

	700 m	800	900	1000	1100	1200	1300	Forest limit 1400	1500	1600
Fagus Sieboldi	████████████████████									
Abies Mariesii ··· ·			── ── ── ── ──				── ────		
Betula Ermani · ·			── ── ──				── ── ──		
Ilex Sugeroki, ssp. brevipedunculata	████████████████████████							── ── ──		
Epigaea asiatica	██████████████████									
Ledum palustre, v nipponicum				────────────				── ── ──		
Pinus pumila								──────────────		
Gaultheria Miqueliana	██████████									
Daphniphora adenothrix		█████								
Empetrum nigrum ·		── ── ── ── ── ── ── ── ──								
Vaccinium Vitis-Idaea		── ── ── ── ── ── ── ── ── ── ── ──								
Loiseleuria procumbens ·								── ──		
Andromeda nana · ··						── ── ──	── ──		
Phyllodoce aleutica						──────────				
Cassiope lycopodes ··					── ── ── ── ──				
Diapensia lapponica, v anatica					── ── ── ── ──				
Geum pentapetalum					──────				
Fauria crista galli ·						────────				
Primula nipponica · ·						── ── ──				
Aquilegia akitensis									──────	
Arnica unalaschensis									──	
Sedum Rhodiola, v congesta									──	
Pinguicula vulgaris ···									──	
Pedicularis frutescens									──	
Campanula lasiocarpa									──	

pure association and invades farther upwards, becoming smaller and smaller, and at last there becoming extinct.   The elevation of this limit is notably different in the different mountain slopes, most probably due to the climatic conditions.
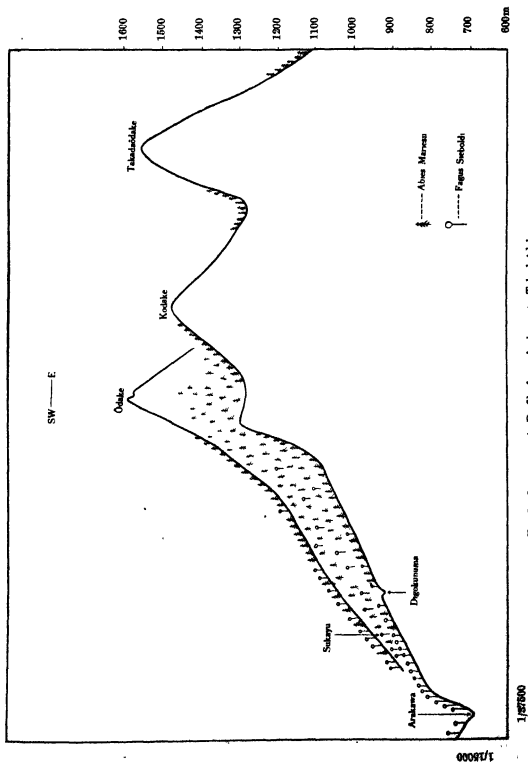
Fig. 3. Diagrammatic Profile from Arakawa to Takadodake.
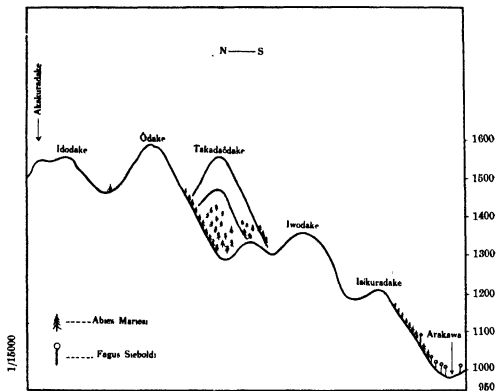
Fig. 4 Diagrammatic Profile from Akakuradake to Arakawa.

2. Shrub zone.

As one ascends above the tree limit of *Abies Mariesii*, there occurs a complete change of physiognomy which makes us feel intuitively that we are really in alpine conditions. So-called "Krüppelgürtel"[1], which is much reported by European ecologists, is not clear in this mountain and generally speaking, the tree limit changes to the shrub zone directly. As is usual in the other alpine regions of Japan[2], in this zone *Pinus pumila*-association is exclusively dominant,

[1] SCHROETER, C. Das Pflanzenleben der Alpen Zürich, p. 38, 1926

[2] HAYATA, B. The Vegetation of Mt. Fuji, Tokyo, 1911.
KOIDZUMI, G. Genetic and floristic phytogeography of the alpine flora of Japan (Japanese)., Bot. Mag. Vol. XXXIII, p. 193. 1919.

attaining to as far as the summit of the highest peak of Ôdake. But
at rocky fields, precipices and hygrophytic localities there observed
numerous species of alpine dwarf shrubs and herbs instead of *Pinus
pumila*. As aleady pointed out, *Pinus pumila* occurs in a far lower
elevation of Digokunuma (870–890 m.), Akamizuzawa (900 m), Suiren-
numa (990 m.), and Kenasitai (1000 m.). This fact shows the distribu-
tion of this plant is based on the edaphic rather than the climatic
conditions and not much influenced by the elevation. Then the
appellation *Pinus pumila*-zone is not exact. It is often observed that
the dispersal of the cone of this plant is carried by a crow (*Nucifraga
caryocatactes japonicus*). The wind is not so great an agency for
the dispersal of the wingless seeds as is usually thought.

EXPLANATION OF THE PLATES.

PLATE XVII

Fig 1   *Rodgersia podophylla, Pelasites japonica* as the underherb of *Pterocarya-
         Alnus hirsuta*-Association July 1929
Fig 2   Kaminota, a swampy part of Kenasitai (after photograph).
Fig. 3   A part of Suirennuma, showing the damage to terminal branches of
         Abies Mariesii by an unfavourable soil condition Peak Takadaôdake
         in the distance
Fig 4   General view of a moor of Senninta at ca 1320 m. *Fauria Crista-galli,
         Geum pentapetalum, Primula nipponica, Coptis trifolia, Narthecium
         asiaticum, Aletis foliata, Eriophorum alpinum, Carex stellulata, Gentiana
         nipponica* are common *Juncus curvatus* is found in shallow pools

PLATE XVIII

Fig. 5.   *Narthecium asiaticum*-Association in a marshy place at Digokuyati at
         ca 960 m
Fig 6   *Eriophorum gracile*-Association at the same habitat as Fig. 5.
Fig 7.   *Lysichton camtschatense*-Association, flowering just after the snow has
         melted
Fig 8   Invation of *Abies Mariesii* into *Pinus pumila*-Association at the alti-
         tude of 1500 m. on the southern side of Ôdake.

PLATE XIX.

Fig. 9   A peak of Inkura (ca 1200 m) showing a mixed growth of *Pinus
         pumila, Abies Mariesii, Rhododendron brachycarpum, Tsuga diversifolia,
         Rh Tschonoskii*, v *typicum*.

Fig 10   A community of alpine dwarf shrubs – *Empetrum nigrum, Vaccinium Vitis-Idaea, Loiseleuria procumbens* & *Pinus pumila* (young) at an altitude of ca 1500 m. on Ôdake.

Fig. 11.   A small community of alpine dwarf plants· – *Cassiope lycopoides, Pinguicula vulgaris, Geum pentapetalum, Diapensia lapponica,* v *genuina* at an elevation of 1500 m. on a rocky slope of Idodake

Fig 12   A small community of *Phyllodoce aleutica* at an altitude of 1180 m on a rock-covered field of Idodake

PLATE XX

Fig 13   *Fauria Crista-galli* & *Primula nipponica* Association at an altitude of ca 1500 m on a exposed spot on Idodake

Fig 14   Part of a *Trollius patulis*-Association mixed with a few *Artemisia integrifolia* on the inner slope of Idodake crater (ca 1500 m)

Fig 15   *Geum pentapetalum* Association an altitude of ca 1550 m Ôdake-crater

Fig 16   A small community of *Pentastemon frutescens* at an elevation of ca 1430 m on a gravel field of Ôdake.
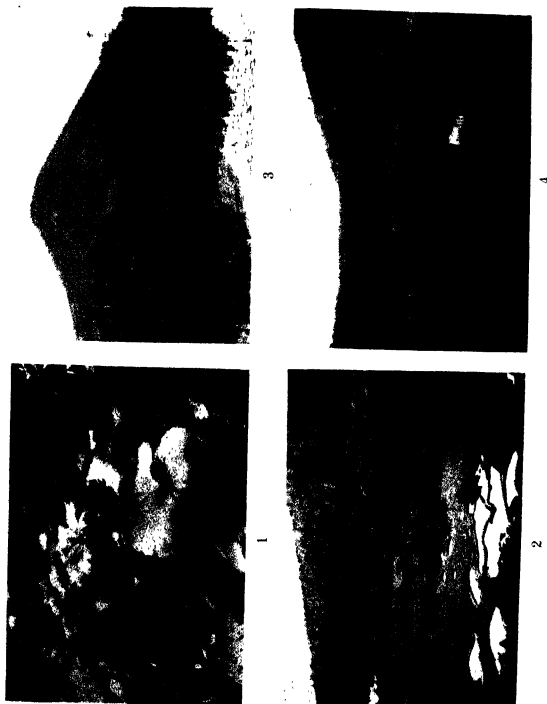
Y. HORIKAWA: Vegetation of Mt. Hakkôda.
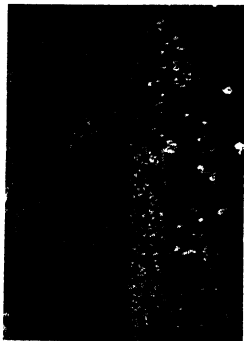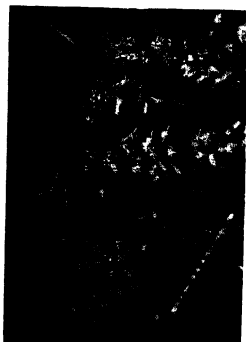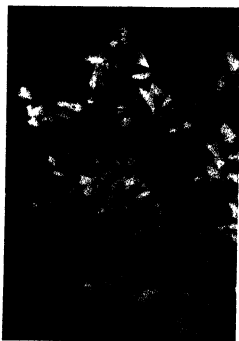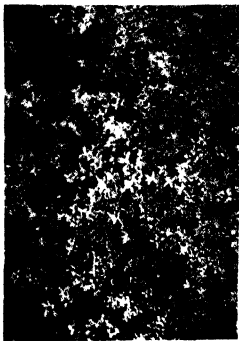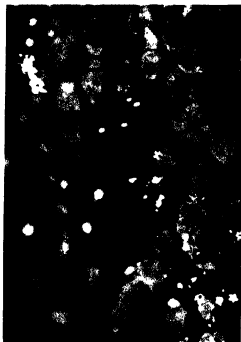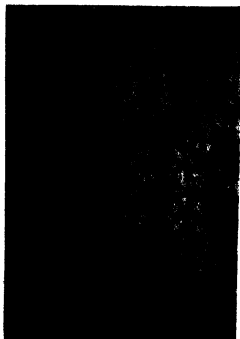
Y. HORIKAWA: Vegetation of Mt. Hakkôda.

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Y. Horikawa: Vegetation of Mt. Hakkôda

Physiological Studies on Drosera

I On the Proteolytic Enzyme of Drosera rotundifolia *

By

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(With 4 Text figures).

I. INTRODUCTION

It is well known that *Drosera* and other so-called insectivorous plants catch insects, but on the biological significance of this capture some lay stress on the protection from the insects and others on the nutritive value to these plants According to the reports of REISS (1878), FR. DARWIN (1880) BÜSGEN (1883) KOSTYTSCHEW (1923) and other authors, the plants grow more healthy when animal food is given them than in other cases. And KOSTYTSCHEW (1926) says that the moorland where these plants grow may be poor in nitrogenous nourishments and mineral salts so that insectivorous plants catch in sects and digest them to supply the nitrogen and mineral salts which are indespensable to them

It is obviously known that the secretion from the leaves dissolves the insects' bodies, while we have yet no particulars about the process of digestion CH DARWIN (1875) observed the conditions of digestion, putting various proteins on the discs of the leaves and trying the effects of many substances, i. e water, acids salts, etc putting them on the leaves or immersing the leaves into them or the solution of them.

REESS and WILL (1875) state that a glycerin extract of the secreted substances of *Drosera* leaves with an addition of weak hydrochloric acid dissolved the swollen-up fibrin in 18 hours at 19–23°C with the result that the digested liquid gave a peptone reaction

TAIT (1875) gathered the secreted fluid of *D* dichotoma with a

feather, and dissolved it in distilled water and precipitated and purified the active principle by means of cholesterin. As the milk was coagulated by the solution of the precipitate, he thought that the precipitate contained a pepsin-like ferment which he called "Droserin".

These authors have believed that the digestion of insects was effected by means of a "ferment", but TISCHUTKIN (1889) and DUBOIS (1890), on the contrary, were of the opinion that the process was due to the action of bacteria.

WHITE (1911) extracted the leaves of *D. auricularia*, *D. Menziesii*, *D. Whittakeri* and *D. peltata* with water and chloroform, and found that the substances precipitated from these filtrates by ammonium sulfate could dissolve fibrin, and gave the products giving biuret reaction. But the further decomposition of peptone was not found. Of course, sterilized apparatus was used throughout his experiment to get rid of the bacterial action.

Further, DERNBY (1917) dialysed the glycerin-extract of leaves of *Drosera rotundifolia* through the collodium membrane, to get the enzyme solution, and tested for the presence of tryptase and ereptase, especially of peptase. In regard to the last one he made digestive experiments in the solution of various acidities, i.e. pH 4, 5 and 6 made by adding N/5 hydrochloric acid, and in all these experiments acid-albumen was applyed at 38°C. As the criterion of the degree of digestion he took the precipitation method by stannous chloride after 3 days' digestion, and found that pH 5 gives the optimum acidity. The tests for tryptase and ereptase fell out negative, so he concluded that enzyme of *Drosera*-leaves is of the pepsin- and of neither the trypsin- nor erepsin-type.

From the above, the digestion of insects seems to be due to a pepsin-like enzyme or one of peptases, but the whole subject of nutrition of nitrogenous substances in the insectivorous plants is not yet certainly settled. The aim of this study is to find out the conditions of enzyme action, especially the acidity, the temperature, and the effects of poisons upon it, and to know what sort of pepsinase or peptase it is.

II. EXPERIMENTS

A) Isolation of Drosera-enzyme.

In this experiment I have used the leaves of *Drosera rotundifolia* which were collected in the vicinity of the Mt. Hakkôda Botanical Laboratory, Tôhoku Imperial University. These leaves excelled in size those from any other localities, and also in the richness of the secretion.

To isolate enzyme I selected the leaves free from insects' bodies or any other foreign matter. About 600 g. of fresh leaves were bruised in the mortar, mixed with 600 cc. of glycerin, 300 cc. of distilled water, and a few drops of toluene, and left for several days at room temperature. The press juice was filtered off, and 1200 cc. of filtrate was obtained, which was reserved in an ice-safe after adding several drops of toluene. This mother solution of enzyme was of a reddish violet colour and also of strong acidity. The enzyme was precipitated as an adhering mass by the addition of acetone from about 10 to 15 times the volume of the enzyme solution, whereby almost all the colouring matters remained in acetone. After the removal of acetone, the powder was dissolved in water to about twice of the volume of the original solution. In the following experiments such solution of enzyme was always used.

B) The Effect of Acid on the Enzyme.

Reess and Will (1875) used an enzyme solution acidified with a few drops of hydrochloric acid and White (1911) added four drops of 0.1% hydrochloric acid to 100 cc. of the neutral enzyme solution. Recently Dernby (1917) examined the enzyme solution acidified with hydrochloric acid, the pH being 4, 5 and 6, and found that at pH 5 the digestion was the strongest. According to the result of these authors the enzyme seems to act best in a weak acidic medium. Therefore I tried to determine the optimum activity of digestion in the solution with acidity: pH 4–7 prepared by the addition of hydrochloric acid as in the experiment of Dernby. And in these cases, carmin-fibrin was used and the temperature was kept at 38°C. Results were negative, i. e., the carmin-fibrin always remained at the bottom

of the test-tube and its colour was dark red even after 24 hours. The reason why the proteolytic action in the extract of *Drosera*-leaves fell negative may probably be found in the presence of some obstacles or paralyzing substances with the enzyme. To get further insight of this point the following experiment was made.

Experiment I.

1 cc. of 0.05% pepsin solution (Pepsins Scales MERCK) was acidified with 3 cc. of N/30 hydrochloric acid and 1 cc. of the *Drosera*-enzyme solution. As the control the solution of NaCl in place of *Drosera*-enzyme was added, as shown in Table I. After 0.01 g. each of carmin-fibrin (GRÜBLER) was suspended in these tubes, they were kept in the thermostat at 38°C., and the digestion was observed at intervals.

TABLE I.

	Drosera-enzyme	N/30 HCl.	0.05% Pepsin-solution
(A)	1 cc	3 cc.	1 cc
(B)	0.85% NaCl 1 cc	„	„

In (A)-solution, as the results of digestion the deep red colour appeared after 30 minutes, while in (B) it was very faint.

These experiments were repeated several times, but in all cases the same results were obtained. The digestion in (A)-tube with the *Drosera*-enzyme solution was always found to be much better than that of the other (B). This result differs from the above one which was got with the acidity of about pH 4–7. It may be caused among other things, for example, by the decomposition of some substances accompanying the *Drosera*-enzyme, but the most probable cause of it is in the strong acidity of the medium. In other words, these experiments have been executed with a strong acidity which was nearly equal to the optimum for pepsin. And the *Drosera*-enzyme may have an optimum acidity stronger than that of most enzymes ever known in plants, so that while at pH 4–7 the reaction was not clear, in an acidity stronger than those above given the more powerful digestion

may probably be caused in co-operation with pepsin. The following experiments show that it is really the case.

Experiment II.

1 cc. of the *Drosera*-enzyme solution and 2 cc. of N/10-N/1000 hydrochloric acid were mixed in each test-tube, and 0.01 g. of carmin-fibrin each was plunged in it, and then it was placed in the thermostat at 38°C. Moreover, as a control, boiled enzyme solution, into which corresponding concentration of acid was added, was prepared, to compare the grade of the digestion through all experiments.

TABLE II.

Number	Enzyme-solution	HCl		Time of observation. (hours)								
				½	1	2	3	4	5	6	16	24
(1)	1 cc	N/10 2 cc		—	—	—	—	—	—	—	—	—
(2)	„	N/100 „		—	—	—	+					
(3)	„	N/1000 „		—	—	—	—	—	—	—	—	—

Remarks: (+) shows that the digestion had commenced and the solution coloured deeper than the control

(—) shows that the digestion had not commenced yet

In the case (1), the digestive action was not noticed and even after 24 hours the fibrin remained untouched. In (2), the colouration appeared stronger than that of the control in 3 hours and the fibrin was completely dissolved and the liquid became opaque after 16 hours, while in the control-tube the fibrin remained undissolved at the bottom of the tube. In (3), there was no remarkable change even after 24 hours.

Experiment III.

As may be seen from the above experiments, the optimum action of the *Drosera*-enzyme takes place very probably in the range of acidity which results from the mixture of 1 cc. of enzyme solution and 2 cc. of N/100 hydrochloric acid. To know further the optimum acidity for the enzyme, combinations of enzyme solution with the

hydrochloric acid solution of different concentrations were prepared.
The results are shown in Table III.

TABLE III.

Number	Enzyme-solution.	HCl		Time of observations (hour).							
			1/4	1/2	1	1¼	2	2¼	3	4	6
(1)	1 cc.	N/20 2 cc	+								
(2)	,,	N/40 ,,	−	−	+						
(3)	,,	N/60 ,,	−	−	−	+					
(4)	,,	N/80 ,,	−	−	−	+					
(5)	,,	N/100 ,,	−	−	−	−	−	−	+		
(6)	,,	N/120 ,,	−	−	−	−	−	−	−	−	−
(7)	,,	N/140 ,,	−	−	−	−	−	−	−	−	−
(8)	,,	N/160 ,,	−	−	−	−	−	−	−	−	−
(9)	,,	N/180 ,,	−	−	−	−	−	−	−	−	−
(10)	,,	N/200 ,,	−	−	−	−	−	−	−	−	−

In (1), the digestion began in 15 minutes and the fibrin was
completely dissolved during 6 hours; in (2) the digestion commenced
in an hour and the fibrin disappeared after 24 hours; while in (3),
even after 24 hours, a few fibrin remained yet. With the decrease
of acidity, the digestive action becomes weaker and weaker, and in
the tube (6) in which the enzyme solution was mixed with N/120
hydrochloric acid the digestion began after 24 hours, and in the
others of weaker acidity was no trace of digestion evident during 24
hours. So the optimum activity of the enzyme must be found in the
more acidic side than that of the test with N/40 hydrochloric acid.

Experiment IV.

Based on the result of the Experiment III, further experiments
were carried on in the range of acidity of N/10–N/100 hydrochloric
acid. The hydrogen-ion concentration of each solution was measured
before and soon after the beginning of the digestion by means of a
potentiometer.

As shown in Table IV and Fig. 1, the optimum action of Drosera-
enzyme is found in the mixture of 2 cc. of N/20 hydrochloric acid
and 1 cc. of enzyme solution, the pH of which is 1.54. Thus, we
can easily say that the optimum action of this enzyme is to be found
in the acidity of about pH 1.5 when fibrin is used. This fact is so

TABLE IV.

Number.	Enzyme-solution.	HCl	pH Before digestion	pH Soon after the beginning of digestion	10'	15'	20'	25'	30'	35'	40'	45'	50'	55'	60'	70'	80'	90'
(1)	1 cc.	N/10 2 cc	1.18	1.19	–	–	–	–	–	–	–	–	–	–	–	–	–	–
(2)	:	N/15 :	1.35	1.37	–	+	–	–	–	–	–	–	–	–	–	–	–	–
(3)	:	N/20 :	1.54	1.4	–	+	+	–	–	–	–	–	–	–	–	–	–	–
(4)	:	N/25 :	1.64	1.65	–	–	+	–	–	–	–	–	–	–	–	–	–	–
(5)	:	N/30 :	1.75	1.78	–	–	–	+	–	–	–	–	–	–	–	–	–	–
(6)	:	N/40 :	1.89	1.90	–	–	–	–	+	–	–	–	–	–	–	–	–	–
(7)	:	N/50 :	1.97	2.03	–	–	–	–	–	+	–	–	–	–	–	–	–	–
(8)	:	N/80 :	2.23	2.30	–	–	–	–	–	–	–	+	–	–	–	–	–	–
(9)	:	N/100 :	2.59	2.40	–	–	–	–	–	–	–	–	–	–	–	–	+	–
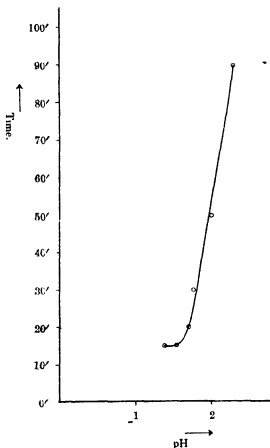
Fig. 1. The effect of acid on the *Drosera*-enzyme

divergent from the optimum acidity of plant enzymes ever known, that the *Drosera*-enzyme may rather rank as an animal pepsin, at least in respect to its optimum acidity.

C) Acids Contained in the Extract of the Leaves.

CH. DARWIN (18 75) observed the effects of some acids, placing drops of them on the discs of leaves, or more commonly, immersing them in the acid solutions, taking as the criterion of their effects the inflection of tentacles and aggregation of plasma. The results he obtained were as follows:

I) Acids which cause inflection but are not poisonous: Formic, hydrochloric, boracic, and malic acids.

II) Acids which cause inflection and are poisonous: Nitric, iodic, hydriodic, sulphuric, phosphoric, acetic, propionic, oleic, carboric, lactic, benzoic, and hydrocyanic acids.

III) Acids which do not cause inflection and are not poisonous: Gallic, tannic, tataric, citric, and uric (?) acids.

REESS and WILL (1875) analysed the fluid secreted from the gland

of *Drosera*-leaves under stimulation with glass-dust, and found the presence of a variety of fatty acids, among which formic acid was recognized with certainty, and from the characteristics of the smell the presence of propionic and butyric acids also were supposed. FRANK-LAND (1875) used the same method as that of REESS and WILL. He analysed the secreted fluid and found that the fluid contained no trace of hydrochloric, sulphuric, tataric, oxalic or formic acids. On the result of the analysis of silver salt he says: "The number obtained, however, corresponded nearly with that of propionic acid, and I believe that this, or a mixture of acetic and butyric acids, were present in the liquid. The acid doubtless belongs in the acetic or fatty series."

It is clear that the acids secreted from the *Drosera*-leaves do not injure their own bodies, so that the acids must belong to that of the first or third group which have been mentioned by CH. DARWIN. On the other hand from REESS and WILL's work it is very probable that formic acid may most likely be found among them.

To determine this point calcium carbonate was added to the above mentioned glycerin-extract of *Drosera*-leaves, filtered off the excess of the calcium carbonate, and from the filtrate the calcium salt was precipitated with absolute alcohol. The precipitate was dissolved in a small quantity of water warmed on the water-bath; this solution was left to evaporate slowly, from which resulted white fine prismatic crystals. With these crystals was made the test of the formic acid reaction in the following methods:

I. A saturated solution of mercuric chloride and a small quantity of natrium acetate were added to the solution to be examined, from which a white precipitate of mercurous chloride was yielded.

II. The solution was warmed with mercuric oxide or silver nitrate, from which metaric mercury or silver was liberated.

III. To about 10 cc. of the solution to be examined, metallic magnesium (0.5 g.) and, drop by drop, 25% HCl (6 cc.) were added while cooling, then a peptone solution (5 cc.) containing ferric chloride was mixed in: when the conc. sulphuric acid (10 cc.) was slowly poured into the mixture the violet colour appeared. (H. FINKE's method).

All reactions gave a positive result. Therefore the evidence is

brought that the leaves contain formic acid.

The meaning of the presence of formic acid in *Drosera*-leaves may be not merely to kill the insects, but to digest them, as it is also the case in some insects to capture the food and to protect their bodies. FRANKLAND said that the conditions were also unfavorable, as it was late in the year and leaves were small". For this reason he could not recognize the formic acid in *Drosera*-leaves.

D) The Effect of the Temperature on the Enzyme.

REESS and WILL, and TAIT had investigated the digestion at room temperature, WHITE at 32° or 38°C and DERNBY at 38°C. These experiments in regard to the effect of temperature on enzyme action were not systematically, but rather optionally carried out, so that the further experiments on this point were necessary to know the characteristics of *Drosera*-enzyme.

Experiment V.

1 cc. of the enzyme solution was made acidic (pH 1.5) by the addition of 2 cc. of N/20 hydrochloric acid. In many tubes this mixture was taken and to each of them 0 01 g. of carmin-fibrin was added. The tubes were immersed in water at 0–60°C. The results are given in Table V and Fig. 2.

TABLE V.

| Number | Temp | Time of observation (minutes). | | | | | | | | | | | | | |
		5′	10′	15′	20′	25′	30′	35′	40′	45′	50′	55′	60′	65′	70′
(1)	0°	−	−	−	−	−	−	−	−	−	−	−	−	−	−
(2)	10°	−	−	−	−	−	−	−	−	−	−	−	−	−	−
(3)	20°	−	−	−	−	−	−	−	−	−	−	−	−	−	+
(4)	30°	−	−	−	−	−	−	+							
(5)	35°	−	−	−	+										
(6)	40°	−	−	+											
(7)	45°	−	−	+											
(8)	50°	−	−	+											
(9)	60°	−	−	−	−	−	−	−	−	−	−	−	−	−	−

At the temperature of 40–45°C., the digestion began in 15 minutes; especially at 40°C. it was most rapid and after 5–6 hours

the carmin-fibrin had completely disappeared, while at 35°, 45° and
50°C. the fibrin remained at the same hour.   At 0°C. it was more
than 20 hours before the digestion began, and at 10°C. 180 minutes.
On the other hand it is evident from the table that a temperature
higher than 55°C. is too high for the digestion.
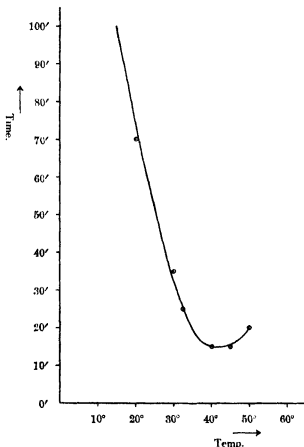
Fig. 2. The effect of temperature on the *Drosera*-enzyme

Comparing the *Drosera*-enzyme to the other proteolytic enzymes,
above all to papaïn which is best known, the digestion product of
the former is the same with that of inactiviated papaïn, but there
are differences between the two, not only in the optimum acidity,
but also in the optimum temperature for them.   So it is remarkable

that *Drosera*-enzyme has also a resemblance to the animal pepsin in respect of the optimum temperature for them.

E) The Products of Digestion.

As already described, it is known that the proteolytic process of the *Drosera*-enzyme goes as far as proteoses and peptones but not further. So I carried on the following experiment to see whether it would be the case or not.

Experiments VI & VII.

For these experiments, the enzyme was mixed with a peptone solution, and hydrochloric acid; the acidity of the medium was adjusted at pH 1.5. For peptone solution WITTE's or KÜHNE's peptone purified by the ordinary method was used. The rotatory power of the mixtures was first estimated soon after the preparation of the mixture by the aid of the SCHMIDT-HAENSCH's polarimeter, then the mixed solutions were kept in the thermostat at 38°C., and from time to time the rotations were noticed As these experiments were carried on only to see the change of rotation, I did not use a given concentration of peptone-solution. The source of light was an incandescent light.

TABLE VI.		TABLE VII.	
WITTE's peptone.		KÜHNE's peptone.	
Time (hours)	Rotatory power	Time (hours)	Rotatory power
0	−15′	0	−23′
⅓	,,	⅓	,,
1	,,	1	,,
2	,,	2	,,
4	,,	4	,,
24	,,	24	,,

As the above two tables show, the rotatory power of WITTE's and KÜHNE's peptones were not changed by the presence of *Drosera*-enzyme. So it is very probable that *Drosera*-enzyme does not hydrolyse peptones and proteoses into amino acids. In this respect also the resemblance of the *Drosera*-enzyme to pepsin is remarkable.

F) The Effect of Poisons on the Enzyme.

So far as the acidity, temperature and products of digestion are concerned, the *Drosera*-enzyme resembles pepsin. So the question arises whether they are really the same or not, a point which is important not only in the physiology of *Drosera*, but also to the question of the intimate relation between the animal and vegetable kingdoms. Therefore the study is made of the relation of the *Drosera*-enzyme to pepsin through the effects of poisons. The effects of poisons on several enzymes, for instance, lipase, esterase etc., have been investigated by many authors, but SMORODINZEW (1924, 1925) and others among them based their results only on the present knowledge of enzymes in their studies. We have yet no conclusive knowledge on this problem, so that here must be described of my studies in some detail. As the full description of the method and results of the experiment on pepsin, however, will be reported in the next paper, the results of the experiment on the *Drosera*-enzyme are given here.

The poison used in this experiment was of two kinds; namely quinine-hydrochloride and atoxyl.

Each given quantity of 0.1% edestin-solution, the solution of poison which beforehand have been warmed in hot water exactly at 39°C. and enzyme-solution were mixed and the total volume was made up to 20 cc. in a flask, soon immersed in water of the same temperature. The acidity of the mixture was kept at the favorable strength. At 20', 40', and 60' respectively after the immersion of the flask, 5 cc. of the mixture was taken out, poured into 15 cc. of cold water in a flask, and finally 0.5 cc. of 40% sulphosalicylic acid was added drop by drop to it while shaking the vessel. The degree of turbidity was determined by means of the KOBER's nephelometer in comparison with the standard solution. The more the digestion progress, the more the turbidity decrease. The standard solution contained half the quantity of edestin of that in the solution examined and had added the same quantity of water instead of the enzyme solution. Moreover, I changed the order of addition of edestin, enzyme and poison in every way. And the effects of each poison in acidic or neutral solutions were also studied. The order of additions is described in the tables, in which the number is given in the unit of mm. In

each series, the digestive mixture containing the same volume of
water, instead of the solution of poison, was taken as the control.
It is worthy of notice that in these experiments, the enzyme pre-
cipitated by acetone was dissolved in the same volume of water as
in the original liquid.

Experiment VIII.

1) The effect of quinine-hydrochloride.

(A) The order of addition   (Enzyme + Edestin) + Quinine-hydro-
chloride. Into a volumetric flask of 20 cc. I measured accurately
with pipettes 5 cc. of enzyme solution, 6 cc. of water warmed to 39°C.,
and 5 cc. of edestin solution of the same temperature. The mixture
was shaken in water of 39°C., and soon had added 4 cc. of 2.5%
quinine-hydrochloride. The concentration of poison in the mixture
was 0.5%.

(B) The order of addition.   (Enzyme + Quinine-hydrochloride) +
Edestin. The method of addition was in general the same as in (A).

TABLE VIII.

Order of addition	Time of observation (minutes)		
	20′	40′	60′
Control	mm 14.5	mm 19.2	mm 23.8
(A)	15.9	22.9	29.4
(B)	14.4	19.1	23.7

As shown in Table VIII and Fig. 3, the digestion in (A) was
evidently accelerated while in (B) neither acceleration nor repression
was noticeable.

The quinine-solution acidified by hydrochloric acid gave the same
results as the above mentioned in its effect on the *Drosera*-enzyme
with respect to the order of addition. On the other hand in pepsin,
quinine-hydrochloride repressed the digestion and such an acceleration
as in the above was not found in any cases of addition.
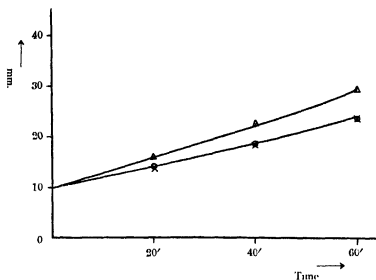
Fig. 3   The effect of quinine hydrochloride on the *Drosera*-enzyme
⊙   ·· Control
△   (A)   · (Enzyme + Edestin) + Quinine
×   (B)   (Enzyme + Quinine) + Edestin

Experiment IX.

2) The effect of atoxyl

The method of experiment and addition was the same as in the above cases, excepting the addition of 2.5% atoxyl in place of quinine-hydrochloride.

(A) The oder of addition. (Enzyme + Edestin) + Atoxyl.

(B) ,,   ,,   ,,   ,,   (Enzyme + Atoxyl) + Edestin.

TABLE IX.

Order of addition.	Time of observation (minutes).		
	20′	40′	60′
	mm	mm	mm.
Control.	14.3 ··	19.0	23.7
(A)	16.7	24.5	34.4
(B)	16.2	23.9	34.2
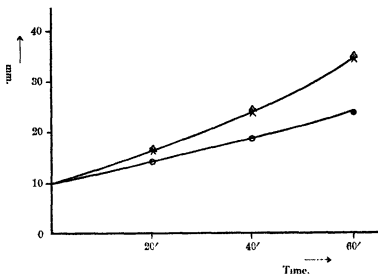
Fig. 4   The effect of atoxyl on the *Drosera*-enzyme
○·   Control
△   (A)   (Enzyme + Fdestin) + Atoxyl.
×   (B)   (Enzyme + Atoxyl) + Edestin

As is evident from Table IX and Fig. 4, the digestion in the
mixture which contained atoxyl was always accelerated, whatever the
order of addition may have been. When pepsin was used acceleration
of the digestion was found in (A), and repression in (B).

As we have seen above, *Drosera*-enzyme resembles pepsin as well
in the optimum acidity and temperature for them as in the products
of digestion, but the former is different from the latter in respect to
the effect of poisons on them. It is, however, known that the effective-
ness of any given enzyme-solution above all depends on the kinds
and quantity of the accompanying substances. So the accompanying
substances must be investigated, if we desire to settle the question
of the specific difference between the two by the effect of poisons.
As above stated the pepsin used in this experiment is a commercial
one (MERCK). So it is very probable that the accompanying sub-
stances in these enzymes differ from each other. Therefore the
question of the specificity of *Drosera*-enzyme remains to be studied
further with purified materials in the future.

III. SUMMARY.

To understand the digestive process of *Drosera*, studies on the proteolytic action of the glycerin-extract of the leaves were carried out, and the results can be summarized as follows:

(1) A powerful proteolytic enzyme exists in the extract of the *Drosera*-leaves.

(2) In a strong acidic medium, *Drosera*-enzyme acts on proteins and hydrolyses them to proteoses and peptones. The optimum activity is found in about pH 1.5, i. e., in such an acidity as is rarely known in any other enzymes of plant origin, and rather resembles that for pepsin.

(3) The optimum temperature for the *Drosera*-enzyme is about 40°C.

(4) In *Drosera*-leaves formic acid is detected.

(5) The effect of poisons on the enzyme is as follows:

    a) Quinine-hydrochloride.

        The order of addition. (Enzyme + Edestin) + Quinine-hydrochloride.

        The digestion is accelerated, while animal pepsin resultes in repression.

        The order of addition. (Enzyme + Quinine-hydrochloride) + Edestin.

        There is no effect, while in pepsin repression occures.

    b) Atoxyl.

        The order of addition. (Enzyme + Edestin) + Atoxyl.

        The digestion is accelerated, as is also the case in pepsin.

        The order of addition. (Enzyme + Atoxyl) + Edestin.

        The digestion is accelerated, but in pepsin it is repressed.

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On the Presumptive Position of the Material of the Medullary Plate in the Frog's Egg, Rhacophorus schlegelii var. arborea (OKADA et KAWANO).*

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(With 6 Text-figures)

INTRODUCTION

In reading GOERTTLER's work " Die Formbildung der Medullaranlage bei Urodeln " which is carried out with VOGT's method of the partial vital-staining, it has seemed desirable to extend the study of this problem to Anuran forms, in which the old theories of Amphibian embryology have been developed. For this purpose, the light-coloured egg of the Anuran species was needed. Fortunately, I heard that the egg of a species of the green frog, Rhacophorus schlegelii var. arborea (OKADA et KAWANO), which has long been famous in Japan for its white egg and its peculiar breeding habits, could easily be obtained from the neighbourhood of the Mt. Hakkôda Botanical Laboratory of the Tôhoku Imperial University in Aomori Prefecture. And in July, 1929, I worked with this species by means of the partial vital-staining method on the problem of from what portion of the egg the medullary plate is formed.

Before going further I wish to acknowledge my indebtedness to Professor E. NOMURA for his much valuable advice and criticisms. Further, I wish to express my hearty thanks to Professor Y. YOSHII, Director of the Mt. Hakkôda Botanical Laboratory, for his kind support of my work during my stay at the Laboratory.

MATERIAL AND METHOD.

In this experiment, the egg of the green frog, *Rhacophorus schle-gelii* var. *arborea* (OKADA et KAWANO), was used. According to OKADA ('28), the breeding season of this species commences towards the end of June in Aomori Prefecture. Eggs are laid on any species of trees on the banks of lake, marsh or pond, or in a hole of grass on the bank. I collected them from the neighbourhood of the labora-tory just before use.

The egg is taken out from the white foamy mass which covers it with fine pincettes, and is separated from most of the gelatinous mass. It is, then, put in a dry glass dish with a cover. A piece of wet filter paper is put on the inner side of the cover. All eggs take their normal orientation in this moist chamber within twenty to thirty minutes. Small pieces of stained agar, which is stained with Neutral red and Nile blue sulphate by VOGT's method, are put on the required point of the surface of the egg membrane with a point of hard hair. The stain of the agar piece diffuses through the thin layer of jelly and the egg membrane, and reaches the surface of the egg cell. In this treatment the egg must be protected from drying by putting it in a moist chamber. After completion of the staining, the egg is washed with water, and the agar pieces are taken off with a point of hair. Observation and sketch are made in a glass box, which is so situated with total reflection prisms that the specimen is observable from a ventral or a lateral side under a vertical microscope without being rotated.

DETWILER ('17) reported that the embryo of *Amblystoma* stained in the capsule for twelve hours in an aqueous solution of 1 : 150,000 of Nile blue sulphate or in a solution of 1 : 400,000 of Neutral red have been kept for twenty-five days after the application of the stain, during which time the development proceeded normally, and at the end of which period all reactions were normal. In my experiment the toxic actions of both stains were very distinct even in six hours' application of the stained agar pieces. The characteristic appearance of the toxic action was, as VOGT ('25) states, shrinkage of cells and delaying of development in the over-stained portion. Three hours' staining at a room temperature of 23° to 25°C gave most satisfactory

results. Marks were kept about twenty-four hours but faded away after fourty-eight hours.

PRELIMINARY EXPERIMENT.

In the frog embryo it was known that the morphogenetic process is always accompanied with the phenomena of the rotation of the egg axis. In this chapter the results of measurement on the rotation will be described. The measurement of the egg was obtained on the basis of ZEISS' Zeichen Prisma sketch of successive stages of the developing egg in using the prisma box. For the measurement I have chosen the following five portions on the surface of the egg: 1) an arc length from the dorsal lip to the posterior margin of the blastocoel*

Fig. 1.

(Fig. 1, and Table 1, A), 2) the longitudinal diameter of the blastocoel

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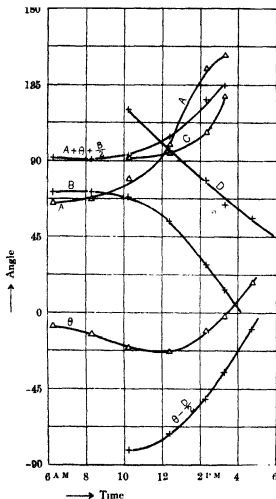
* IKEDA ('02) has pointed out, that in this species the blastocoel is easily observable from the surface through the roof of the egg because of the complete absence of melanin in the egg.

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(B), 3) the arc length between the ventral lip and the anterior margin of the blastocoel (C), 4) the diameter of the yolk-plug (D), and 5) the distance from the dorsal lip to the posterior equator of the egg (θ). Two other measurements, the distances from the anterior equator to the centers of the blastocoel ($\theta - \dfrac{D}{2}$) and the yolk-plug ($A + \theta + \dfrac{B}{2}$), were obtained from A, B, D and θ by calculation. Figure 1 and Table 1 show the results of this measurement, in which the arc length is given in the magnitude of central angle.

At 6.20 A.M. the first trace of dorsal lip appeared. At 10.15 A.M. the blastopore showed a complete circle, and after this time all measurements were obtained. At 3.30 P.M. the blastocoel disappeared, and measurements A, B and C ended at this time. In the values of θ, $\theta - \dfrac{D}{2}$ and $A + \theta + \dfrac{B}{2}$, the positive or negative sign shows the upward or downward direction of measure from the posterior equator.

TABLE 1.

	A	B	C	D	θ	$\theta - \dfrac{D}{2}$	$A + \theta + \dfrac{B}{2}$
6.20 A M 12/VII	64°20'	71°40'	—	—	− 7°50'	—	92°20'
8.20 ,, ,,	67°40'	71°50'	—	—	−12°50'	—	90°55'
10.15 ,, ,,	79°20'	69°00'	91°30'	120°20'	−20°40'	−80°50'	93°10'
0.25 P M ,,	99°50'	54°30'	94°30'	96°20'	−23°00'	−71°10'	104°08'
2.20 ,, ,,	145°14'	28°50'	106°40'	79°20'	−10°44'	−50°24'	148°55'
3.20 ,, ,,	152°34'	14°10'	128°20'	64°50'	− 2°34'	−35°00'	157°05'
4.45 ,, ,,	—	—	—	56°20'	+18°30'	− 9°40'	—

The results are summarized as follows: The first trace of the dorsal lip appears at about eight degrees below the equator. The center of the yolk-plug never goes anteriorly beyond· the lower pole of every stage. It goes rapidly towards the posterior equator after the complete circle of the blastopore is formed. The center of the blastocoel proceeds rapidly towards the anterior equator after completion of the circular blastopore. The area of the blastocoel becomes smaller and smaller as the center advances further and disappears

just before reaching the anterior equator. In every observed stage of development the centers of the blastocoel and of the yolk-plug lie approximately in antipole, and move at nearly equal tempo and direction

EXPERIMENT I

At the beginning of gastrulation the upper pole of the egg was marked with four colour spots around the upper pole. Five hours after marking they incline anteriorly. But their relative position to the center of the blastocoel does not vary. (Fig. 2, a). Ten hours after marking the medullary plate appears on the upper side of the egg and the colour spots rotate anteriorly more than 90 degrees. After twenty hours, in which the neurulation process has been completed, the four colour spots are on the antero-ventral side of the embryo (Fig. 2, b). In every case there is found some space between the anterior end of the head and the area of colour spots. The medullary plate itself has never been derived from the stained area.
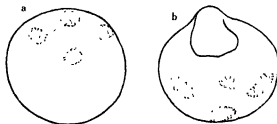
Fig. 2. Marking of the upper pole in the early gastrula. Anterior view. a -- five hours after marking, a small blastocoel is in the center of four colour spots. b -- twenty hours after marking.

The results of this experiment are as follows: The material of the upper pole of the early gastrula moves on anteriorly in the course of development. This movement coincides perfectly with that of the blastocoel while the blastocoel exists. The anlage of the anteromost part of the medullary plate lies between the dorsal lip and the upper pole of the early gastrula, as likewise in the blastula, but it is never derived from the material of the upper pole or of the more anterior portion of this stage.

EXPERIMENT II.

In the blastula stage four colour spots were marked at equal distances on the equatorial zone. The first trace of the dorsal lip appears within the zone of these marks a little below the equator. Three hours after marking (Fig. 3, b), at the stage of 9 A.M. in Figure 1, the dorsal lip grows in crescentic form and acrosses two or three of the marks. Seven hours after (Fig. 3, c), at the stage of 12 A.M. in Figure 1, the blastopore becomes circular, and the blastopore lip cuts all the colour spots. But comparing with the original position of the colour spots, it is evident that the level, in which the groove of blastopore lip appears, is about equal in any side of the blastopore. In the case where the spots are marked somewhat upward, only a small area of colour spot is taken in on the yolk portion, and in this stage the colour spots of the yolk portion have covered with a marginal portion of blastopore lip. After eleven hours (Fig. 3, d), at the stage of 2 P.M. in Figure 1, the four colour spots become longer than in the preceding stage. In many cases the lower area of the colour spots is covered with the blastopore lip. And, moreover, even in the upper part of the colour spots, when it is marked somewhat downward, the deeply stained portion has already been rolled into the blastopore, and only the diffused part of this colour spot remains on the surface of the blastopore lip. After sixteen hours (Fig. 3. e, f), at the stage of 5 P.M. in Figure 1, the four colour spots show a very remarkable elongation. The intensity of stain becomes very feeble, which is caused by the extention of the area of the colour spots. All the colour spots arrange radially around the yolk-plug. The distances between them are equal at the margin of blastopore. At the free end of the marks, however, they show a slight tendency to approach the upper side of the egg at this stage. The plane containing the free ends of the marks has been rotated about 90 degrees from the initial position in antero-ventral direction. The process of inclination of this plane is the same in direction, in tempo and in magnitude as the inclination of the centers of the blastocoel and of the yolk-plug.

The results of this experiment are as follows: In all sides of the blastopore the first trace of invagination appears at the same
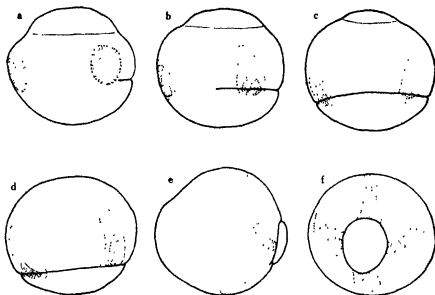
Fig 3 Marking of the equatorial zone at the blastula stage. a — e, side view f, posterior view. a appearance of the dorsal lip, b - 3 hours after marking; c 7 hours after; d 11 hours after; e 16 hours after, f the same

level of the surface of the blastula. In the course of gastrulation the colour spots show a very remarkable elongation in meridional direction in relation to the vertical axis of the blastula stage. The material of the narrow ring zone lying just above the level of invagination is involved beneath the blastopore lip. The remaining portion of the four colour spots are arranged radially around the yolk-plug at equal distances. The blastopore lip converges equally from all side to the yolk pole. The fusion of the material of the lateral lips at the dorsal lip in not observed. The process of inclination of the plane containing the free ends of the four marks is exactly the same as the inclination of the centers of the blastocoel and of the yolk-plug.

EXPERIMENT III

At the end of the blastula stage four colour spots are marked at equal distances close by the outline of the blastocoel (Fig. 4, a and

5, a). The zone limited by these four marks occupies the area be-
tween the pole zone observed in Experiment I and the equatorial
zine observed in Experiment II. Three hours after the appearance
of the dorsal lip (Fig. 4, b and 5, b), at the stage of 9 A. M. in
Figure 1, the level of the colour spots shows a slight tendency of
inclination.
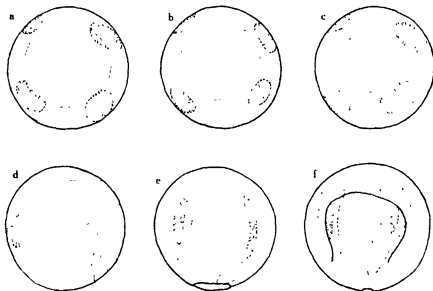
Fig. 4. Marking of the middle zone of the upper hemisphere in the blastula
stage, dorsal view   a — a trace of the dorsal lip appears, a large blastocoel is
observed through the cell layer of the upper pole,   b - -3,30 hours after the
appearance of the dorsal lip;   c — 8 hours after,   d - 12 hours after, just before
the disappearance of the blastocoel, the inclination of the egg axis and the
elongation of the colour spots are seen'   e -17 hours after.   f - 23 hours
after, the medullary plate stage

Eight hours after (Fig. 4, c and 5, c), at the stage of 12 A. M.
in Figure 1, the inclination has progressed. Twelve hours after (Fig.
4, d and 5, d), at the stage of 2 P. M. in Figure 1, one or two
anterior colour spots come to the lower hemisphere of the present
stage, and hence only two or three colour spots are visible in the
upper hemisphere. This fact shows the inclination of the original
vertical axis of the egg. A small blastocoel lies, similar to the
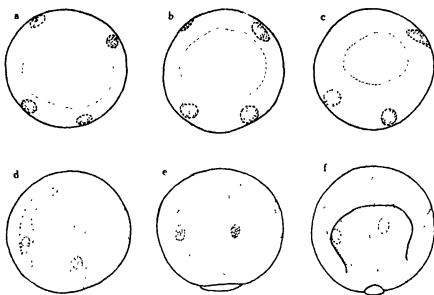
Fig. 5   The explanation is the same as that in Fig 4

preceding stage, in the center of the four marks. All the colour
spots show a remarkable elongation in meridional direction in respect
to the axis through the centers of the blastocoel and the yolk-plug.
Seventeen hours after (Fig. 4, e and 5, e), at the stage of 5 P. M.
in Figure 1, the four elongated colour spots are arranged in the
meridional direction to the horizontal axis of this stage. Two or
rarely three colour spots lies on the surface of the upper hemisphere
of the present stage and the other two on the lower hemisphere.
Twenty-three hours after (Fig. 4, f and 5, f), the egg shows a very
distinct outline of the medullary plate. A small blastopore lies just
below the posterior equator. The four colour spots show no change
in length in comparison with the preceding stage. In most cases,
the upper two colour spots are found in the area of the medullary
plate. The anterior ends of these two colour spots protrud from the
anterior margin of the medullary plate. But in other cases the former
are just on the margin of the latter. The distance between the
colour spots in the medullary plate is somewhat shorter than in the
other place. In other words, the colour spots have travelled to the

dorsal median line of the egg. The posterior end of the four colour
spots are never involved in the blastopore and are laid close by the
blastopore, even in the colour spots which have been taken in the
medullary plate.

The results of this experiment are as follows: In the process of
gastrulation the material of the middle zone of the upper hemisphere
of the blastula stage elongates equally in meridional direction in re-
spect to the vertical axis of the blastula. And at the end of gastrula
the material of this zone covers most of the lower hemisphere. In
other words, the material of the polar area, which is studied in
Experiment I, and the middle zone of the upper hemisphere studied
in this experiment form most of the ectoderm. The medullary plate
is derived only from the material of the limited area in the upper
hemisphere at the side of the dorsal lip. The anterior margin of
this area nearly reaches to the upper ends of the colour spots of this
experiment. And the width is approximately represented by the dis-
tance between the outer margin of two colour spots. In the beginning
of neurulation the presumptive material of the medullary plate con-
densates from both sides to the dorsal median line of the embryo.
At the end of gastrula the posterior ends of the four marks are laid
close to the blastopore, but they are not involved in the blastopore,
and even in the case when the colour spots is on the median line of
the medullary plate the distance between its posterior end and the
blastopore lip is the same as in the other spots.

                            DISCUSSION

In the preliminary experiment it became clear that the blastocoel
and the yolk-plug are in antipole of the egg throughout every stage of
gastrulation. And in the Experiment I, II and III the zones of colour
spots showed same tendency of inclination in direction, tempo and
magnitude with respect to the centers of the blastocoel and the yolk-
plug. Therefore, knowing on the movement of the colour spots, it
is possible to learn the dimention of inclination of the original vertical
egg axis, which coincides with the axis through the centers of the
blastocoel and the yolk-plug. All the results of the experiments gave
the same value in regard to the rotation phenomena of the original

vertical egg axis; that is about 90 degrees in the direction of the anterior equator.

In Experiment II, I have described the phenomena of involution of the surface substance of the equatorial zone into the blastopore. Although in my experiment the area of involution was not accurately determined because of the narrow width in comparison with the diameter of the colour spots, it is conceivable that the upper margin of this area is laid close by the equator. For the reason, in Experiment III, a small portion of the colour spots is always left behind in the ectoderm without being involved even up to the stage of complete closure of the blastopore. Therefore, we come to the conclusion that in this species the ectoderm is derived only from the substance in the upper hemisphere of the blastula.

From the results of Experiments II and III, the presumptive area of the medullary plate can be approximately determined. The anterior end of the medullary plate reaches close to the upper margin of the colour spots of the middle zone, observed in Experiment III. The height of this point measures three-fifths of the radius of the egg upward from the equator. From Experiment III we know that the presumptive area of the medullary plate covers at least two colour spots. The width of this range

Fig 6 Schema of the anlage in the blastula stage, P M M the area of the presumptive medullary material, e – equator, b — the level of the blastopore lip

corresponds to the arc length of 120 degrees in the central angle. Here we come to the conclusion, that the presumptive area of the medullary plate, which is laid down above the dorsal lip, is in the zone bounded by the equator at the base, and by the level of three-fifths of the height of the egg radius from the equator to the upper margin, and by two meridians including the range of an 120° central angle at the sides (Fig. 6). In this area the presumptive medullary material is so

orientated that the anterior margin of the actual medullary plate corresponds to the upper margin of this area, the lateral sides to the meridians, and the posterior end to the equator.

In the course of gastrulation the presumptive area of the medullary plate changes its form in two different directions. The one is the extention of the area in a meridional direction. This process progresses very slowly at the start, but it becomes rapid after the complete circle of the blastopore is formed (Experiments II and III). The other is the condensation, or "Schweckung" (GOERTTLER '25), of the material from both sides to the dorsal median line of the embryo. This process begins after the disappearance of the blastocoel, and it is confined only to the dorsal side of the embryo (Experiment III, Fig. 4, f).

In short, the results of my experiment were favourable to the theory of convergence. The concrescence of the blastopore lip was not observed. The whole material of the medullary plate is laid down in an area just above the dorsal lip in a compressed form. Therefore, the posterior end of the medullary plate is already determined in the position just above the dorsal lip at the beginning of gastrulation. The thick cell layer above the medullary plate is formed only by the condensation of cells from both sides. Of course, the mentioned processes of morphogenesis must be applied only to the normal development of this species, and the regulatory power of parts of the egg in any varied condition is beyond the scope of this experiment.

In 1888, ROUX observed that an exovate, marked on the animal pole of the blastula with a needle, shifts to the ventral surface of the embryo. And he came to the conclusion, "dass die mittleren Furchungskegeln der schwarzen oberen Hemisphäre, also der sogenannten animalen Poles, der Morula und Blastula die Bauchgegend des Embryo aus sich hervorgehen lassen." After this the same experiment was carried out in Anuran embryos by MORGAN and TSUDA ('94), EYCLESHYMEY ('98), KING ('01), IKEDA ('02) and in Urodera by BURFURTH ('93) and EYCLESHYMER ('98). The results of these authors agreed with ROUX's. And later, the same problem was studied with the method of partial vital staining by GOODALE ('11) in *Spelerpes* and by GOERTTLER ('25) in *Triton*. The results obtained

in these Uroderan species differ from the above mentioned authors.
In *Spelerpes* and *Triton* the material of the upper pole is always
taken in on the medullary plate. My results differ from the last two
authors' in spite of the same method of experiment. I believe that
this difference is due to the specificity of the observed species. In
Anura the position of the anterior end of the presumptive medullary
material is much lower than in *Triton* and *Spelerpes*.

On the presumptive position of the posterior end of the embryo
there were two opinions to the present. According to one opinion
it is considered that the posterior end is laid down on opposite side
from the dorsal lip of the egg. ROUX ('88) came to this view for
the first time from his observation of a monster, *Asyntaxia medullaris*,
of the frog. After this, O. HERTWIG ('92), MORGAN and TSUDA ('94)
supported ROUX's conception on the bases of observations on *Spina
bifida* embryo which is formed in natural or artificial conditions. In
1894, MORGAN studied this problem with experimental methods. If
the dorsal lip is stuck with a sharp, cold needle at the time of its
appearance, a monster with a V-shaped neural fold is formed. And
when the embryo is stuck just in front of the dorsal lip i. e., in the
black cell of the region, injury was found later in the cross connective
at the anterior end of the medullary plate. From these results he
came to the same conclusion. But in the other opinion the posterior
end of the embryo is derived from the upper portion of the dorsal
lip. In 1898, EYCLESHYMER studied this problem with the puncturing
method in the frog. His conclusion was that an area lying just above
the point at which the blastopore lip first appears, later forms a
portion of the posterior end of the embryo. The last differs very
much from MORGAN's results, in spite of the use of the same method.
But as IKEDA ('02) has pointed out, it must be a dangerous thing to
rely too much upon the puncturing method, because of the inconstancy
of the intensity of injury. Recently, GOERTTLER ('25) pointed out
with the method of partial vital staining in *Triton*, *Pleurodeles* and
Axolotle, that the posterior end of the presumptive area of the
medullary ·plate is laid down just above the equator at the side in
which the dorsal lip appears. Comparing this with my experiment
in *Rhacophorus*, the position of the area and its morphogenic process
are similar to the principle of the process in *Triton*, *Pleurodeles* and

Axolotle of GOERTTLER's experiments. But with this principle the *Spina bifida*, or the ring embryo in the extreme form are not explained. These abnormal forms may be derived, I think, as the results of regulation in abnormal conditions. Further observation will be needed on this point.

SUMMARY

1. In the embryo of *Rhacophorus schlegelii* var. *arborea* (OKADA et KAWANO) the ectoderm is derived from the substance of the upper hemisphere of the blastula.

2. The presumptive area of the medullary plate is laid down above the dorsal lip of the egg.

3. The presumptive area of the medullary plate is in the zone bounded by the equator at the base, and by the level of three-fifths of the height of the egg radius from the equator to the upper margin, and by two meridians including the range of an 120° central angle at the sides.

4. In this area the presumptive medullary material is so orientated that the anterior margin of the actual medullary plate corresponds to the upper margin of this area, the lateral sides to the meridians, and the posterior end to the equator.

5. In the course of gastrulation the presumptive area of the medullary plate changes its form in two directions; the extention in a meridional direction and the condensation, or "Schweckung", from both sides to the dorsal median line.

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    CVI.

Studien über die Mykorrhiza-Pflanzen im Solfataren-Gebiete auf dem Berg Hakkoda.[1]

VON

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(Mit 6 Text Figuren)

Wie bekannt, findet man Mykorrhiza fast immer an den Wurzeln der auf humusreichen Böden wachsenden Bäume. Neuerdings bemerkte aber FABER[2] Mykorrhiza auch bei allen Pflanzen, die im humusarmen Solfataren-Gebiete in Java verbreitet sind, und vermutete, dass die Mykorrhiza in stickstoffarmen Böden für die Bindung freien Stickstoffs eine grosse Rolle spielt.

Ich habe vor allem feststellen wollen, ob Mykorrhiza auch in unseren einheimischen Solfataren-Gebieten vorkommt, und habe ihre morphologischen Eigenschaften eingehend studiert.

Vorliegende Untersuchung wurde im botanischen Berglaboratorium auf dem Hakkoda unter Leitung Prof. Dr. YOSHII ausgeführt An dieser Stelle möchte ich meinem verehrten Lehrer meinen Dank für seine freundliche Unterstützung und Anregung ausdrücken.

Zur Untersuchung dienten fast alle Pflanzen, die im Solfataren-Gebiete wachsen. Wegen der hohen Azidität des Bodens kommen hier nur verhältnismässig wenige Pflanzen vor. Ich untersuchte 28 bei diesem Standort gesammelte Pflanzen.

Zuerst wurden mit Pilzen versehene Wurzeln, besonders junge, reichlich Pilzfäden besitzende Wurzelspitzen, sorgfältig ausgegraben und mit Wasser gewaschen. Das Material wurde mit Rasiermesser geschnitten und die mit vielen Pilzfäden versehenen Schnitte mit DELAFIELD-Hämatoxylin oder Säurefuchsin gefärbt.

Von diesen 28 Arten konnte ich bei folgenden 6 Arten keine Mykorrhiza finden: Polygonum sachalinense, FR. SCHM., Phragmites

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communis, TRIN., *Hydrangea paniculata*, SIEB., *Juncus curvatus*, BUCH., *Aletris foliata*, FRANCH. und *Pteridium aquilinum*, (L.) KUHN. Obwohl ich bei einigen von diesen Pflanzen wenige Pilzfäden auch auf der Wurzeloberfläche gesehen habe, so kann man doch dabei noch nicht von Mykorrhiza sprechen, weil die Pilzfäden der eigentlichen Mykorrhiza die Wurzeloberflache dicht umhüllen oder sogar ins inneren Geweben eindringen müssen.

Die Mykorrhiza in den anderen 22 Arten lässt sich nach ihren Formen, wie folgt, einteilen.

Typus I. Ektotrophe Mykorrhiza.

1) *Zusammengesetzte Mykorrhiza*, welche die viele Wurzel zusammen umhüllt.

Korallenform-Mykorrhiza. Dieser Typus kommt bei *Pinus pumila* vor (Fig. 1. a. b. c.). Bei dieser Pflanze ist die Wurzel, die sich regelmässig wie eine Gabel verzweigt (Dichotomie), vom Pilzmantel umgeben. Das Wurzelwachstum dieser Kiefer ist im allgemeinen langsam, aber die Wurzeln in den Wurzelmasse verzweigen sich sehr häufig, wodurch sich die Masse vergrössert. Die Pilzfäden erfüllen die Zwischenräume dieser verzweigten Wurzeln, bis sich endlich eine dichte Wurzelmasse bildet.

Die Pilzfäden sind gewöhnlich fast farblos, ein schwarzer Fleck tritt selten auf der äussersten Pilzschicht des Mantels auf, und deren Pilzfäden haben ziemlich verdickte Zellwände. Schon MASUI[1] hat bei
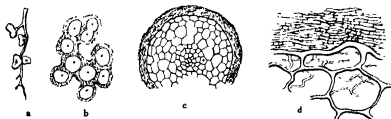
Fig 1   Korallen-Form der Mykorrhiza von *Pinus pumila*, REGEL. (a. b. c).
   a) Äussere Gestalt ca ×5.
   b) Querschnitt durch eine Mykorrhizamasse.
   c) Querschnitt durch eine von einem Pilzmantel umhüllte Wurzel.
      Heterotrophe Mykorrhiza von *Pinus pumila* (d., Erklärung in S. 601).

[1] MASUI, K. (1926), A study of the mykorrhiza of woody plants. Mem. Coll. Sci. Kyoto Imp Univ., Ser. 3., No. 2. pp. 177.

seiner Untersuchung der Mykorrhiza einiger Bäume darauf hinge-
wiesen, dass verschiedenartige Pilze in ein und derselben Pilzmasse
gefunden werden. Die morphologische Verschiedenheit dieser Masse
ist wahrscheinlich auch auf zweierlei verschiedene Pilze zurückzuführen

 2) *Einfache Mykorrhiza*, die eine einzige Wurzel umhüllt.

 A) *Mykorrhiza*, die im Interzellularraum des Wurzelgewebes Pilz-
fäden besitzt. (Fig. 2. a. b. c.).

Dieser Typus kommt bei *Betula Ermanni*, CHAM., var. *communis*,
KOIDZ., *B. Maximowicziana*, REGEL. und *Salix Reinii*, FR. et SAV.
vor. Die Pilzfäden hüllen nicht nur die jungen Wurzeln ein, sondern
dringen auch dicht zwischen die Interzellularräume der Epidermiszellen
ein. Dadurch werden die nebeneinander liegenden Epidermiszellen
später voneinander getrennt und sind nur noch an innere Seite zu
Rindenzellen verbunden. In dieser Weise wird das sogenannte
Hartigsche Netzwerk gebildet Solche Mykorrhiza sieht wie eine
endotrophe Mykorrhiza aus, wenn man sie durch die Zellwand sieht.
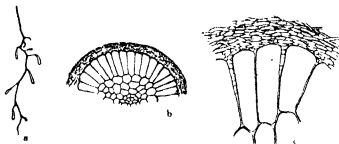
Fig 2 Ektotrophe Mykorrhiza Typus I 2 A von *Salix Reinii*, FR et SAV
 a) Äusser Gestalt. ca. ×3
 b) Querschnitt mit radial gestreckten Epidermiszellen
 c) Ein Stück des vergrösserten Querschnittes, zeigt Hartigsche Netzwerk

 B) *Mykorrhiza*, die nur auf den Wurzel-Epidermiszellen Pilzfäden
besitzt, (Fig. 3. a. b. c.).

Die Pilzfäden umhüllen auch eine einfache Wurzel, aber dringen
nicht in die Interzellularräume ein. Bei einer gewissen Mykorrhiza
ist dieser Pilzmantel dünn und umhüllt die Wurzel dicht und er-
scheint daher wie eine dünne Schicht einer schleimigen Substanz.
Es ist bemerkenswert, dass die Pilzfäden dieser Mykorrhiza sehr
fein sind.

Fig. 3   Ektotrophe Mykorrhiza Typus I 2 B von *Pinus pumila*, REGEL.
   a) Äussere Gestalt ca ×3
   b) Querschnitt durch den Pilzmantel
   c) Ein Stück des vergrösserten Pilzmantels und der Epidermiszellen

TYPUS II.   HETEROTROPHE MYKORRHIZA.

Diese Mykorrhiza wurde nur bei *Pinus Pumila* beobachtet. Die Pilzfäden dringen nicht nur in die Interzellularraume und ins Zellinnere, sondern bilden auch einen Pilzmantel auf der Oberfläche der Wurzel, ganz wie ektotrophe Mykorrhiza. Die Pilzfäden in den Interzellularräumen sind feiner, als die auf den Wurzelepidermiszellen. Obwohl die Pilzfäden auch ins inneren Gewebe eindringen, unterscheiden sich ihre morphologischen Eigenschaften doch von denen der endotropen dadurch, dass die Zellen spärlich mit Pilzfäden erfüllt sind, die nur in den Epidermiszellen und den ihren naheliegenden Rindenzellen verlaufen. Diese Mykorrhiza scheint mir eine Übergangsform von der ektotrophen zur endotrophen Mykorrhiza zu sein (Fig. 1. d).

TYPUS III.   ENDOTROPHE MYKORRHIZA.

1) *Mykorrhiza*, bei der Pilzfäden nur in Epidermiszellen vorkommen. (Fig. 4. a. b. c).

Eine eigentümliche Form kommt bei fast allen Pflanzen von Ericaceen vor; wie *Diplycosia adenthrix*, NAKAI, *Epigæa asiaetica*, MAXIM., *Gaultheria Miquiliana*, TAKEDA, *Leucothoe Grayana* MAXIM. var. *Maximowiziana*, TAKEDA, *Menziesia pentandra*, MAXIM., *Menziesia purpurea* MAXIM., *Vaccinium Axillare*, NAKAI, *V. hirtum*, THUNB., *V. Vitis-Idaea*, L., bei *Shortia soldaneoides*, MAKINO, var. *genuina*, MAKINO, f. *typica*, MAKINO, und *Empetrum nigerum*, L. Bei dieser Mykorrhiza finden sich die Pilzfäden nur in den Epidermiszellen, wo

sie eine fast verwirrte, leicht gelbliche Masse bilden. Solche Zellen
befinden sich besonders reichlich in der Nähe der Wurzelspitze.
Ausserdem verlaufen öfters in derselben Zelle noch andere farblose
Fäden. Diese zweiartigen Fäden, gelb und farblos, gehören wahrschein-
lich zu zwei ganz verschiedenen Arten. In den von der Wurzelspitze
etwas entfernt liegenden Zellen kann man keinen Pilzfaden mehr finden.
Das ist wahrscheinlich auf die Verdauung des Pilzinhalts durch die
Wirtpflanzen zurückzuführen. Diese Zellenreste lässen sich mit
DELAFIELD-Hämatoxylin stark färben. Später verschwinden diese eine
immer kleiner werdende Masse enthaltenden Epidermiszellen, und nun
nehmen die Rindenzellen die äusserste Zellschicht der Wurzel ein.
Auf diesen äusseren Rindenzellen verlaufen wieder viele, neue und
etwas verdickte Pilzfäden. Dabei gibt es aber weniger in die Rinden-
zellen eindringende Pilzfäden, als in den Epidermiszellen vorhanden
sind.
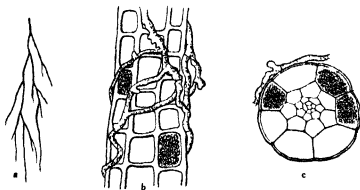
Fig. 4. Endotrophe Mykorrhiza. Typus III. 1 aus *Menziesia pentandra*,
MAXIM

    a) Äussere Gestalt. ca. × 20.

    b) Auf der Wurzel verlaufende Pilzfäden, zeigt auch Pilzmassen

    c) Querschnitt dieser Mykorrhiza; zeigt Pilzmassen in Epidermiszellen

  2) *Mykorrhiza*, bei der Pilzfäden nur in Rindenzellen vorkommen.
    (Fig. 5. a. b. c.)

    Diese Form findet sich in den Arten verschiedener systematischer
Familien: Gramineen (*Miscanthus sinensis*, ANDERSEN, *Molinia
japonica*, HACK., und *Sasa paniculata*, MAKINO et SHIBATA), Capri-

foliaceen (*Vibrunum furcatum*, BLUM.), Aquifoliaceen (*Ilex Sugenoki*,
MAXIM. subsp. *brevipedunclata*, MAKINO) und Liliaceen (*Maianthemum
Convallarea*, WEGG. et ROTH). Bei dieser Mykorrhiza kommen die
Pilzfäden nur in Rindenzellen und nicht in Epidermiszellen vor. Die
in eine Zelle eindringenden Pilzfäden sind gewöhnlich sehr verdickt
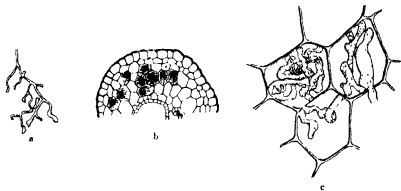
Fig. 5 Endotrophe Mykorrhiza Typus III 2 aus *Molinia japonica*, HACK
  a) Aussere Gestalt ca. ×3
  b) Querschnitt, zeigt Pilzmasse in Rindenzellen.
  c) Vergrösserter Querschnitt, zeigt Pilzfäden in Rindenzellen
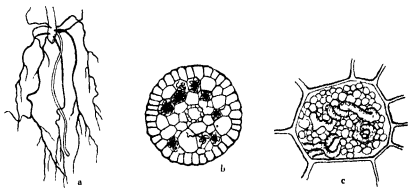
Fig 6 Endotrophe Mykorrhiza Tyus III. 2 aus einer einjährigen Pflanze
    von *Maianthemum smilacium*, A. GREY.
  a) Aussere Gestalt. ca. ×1.
  b) Querschnitt; zeigt einige Pilzmassen in Rindenzellen.
  c) Vergrösserte Rindenzelle mit Pilzfäden und einer grossen Menge
    von Stärkekörnchen.

und bilden da eine ziemlich feste und verwirrte Masse   Wir können
öfters den Kern der Zelle an der zentralen Stelle erkennen, wo die
Pilzfäden sich sammeln.

WURZELBAU DER MYKORRHIZA

Der Wurzelbau ist ganz verschieden, je nach der Verschiedenheit
des Mykorrhiza-Typus. Er lässt sich in zwei Typen unterscheiden.

1) Wurzelbau der ektotrophen Mykorrhiza.

Bei der zusammengesetzten Mykorrhiza stellen die mit Pilzfäden
umhüllten Wurzeln eine regelmässige, gabelförmige Verzweigung
(Dichotomie) dar, aber ihre Entwicklung ist schlecht, und zwar bleibt
der Zentralzylinder nur im jungen Zustande, während die Rindenzellen
etwas grösser als normal sind. Bei der Mykorrhiza des Typus I. 2. A.
lässt sich auch eine auffallende Veränderung in den Epidermiszellen
erkennen ; diese Zellen strecken sich in radialer Richtung aus. Diese
anormale Entwicklung ist wahrscheinlich darauf zurückzuführen, dass
die Pilzfäden zwischen alle jungen Epidermiszellen in der Nähe der
Wurzelspitze eindringen und das HARTIGsche Netzwerk bilden. Dadurch
wird das Längenwachstum der Epidermiszellen gehemmt, die nur bloss
noch in radialer Richtung wachsen können. Eine verhältnismässige
Verdickung der Wurzel dieser Form verursacht wahrscheinlich auch
die radiale Ausdehung der Epidermiszellen. Keine Veränderung des
Gewebes im Typus I. 2. B. lässt sich erkennen.

Bei heterotrophischer Mykorrhiza kann man das Eindringen der
Pilzfäden in die Zellen beobachten, doch lässt sich keine besondere
Veränderung des Gewebes erkennen, obwohl sich die Epidermiszellen,
in denen wenige Pilzfäden vorkommen, vergrössern.

Kurz, auffallende Vergrösserung der Epidermiszellen und der
naheliegenden Rindenzellen ist das Kennzeichen der ektotrophen
Mykorrhiza, während die Entwicklung des Zentralzylinders schlecht
und die Zellteilung in der Wurzelspitze auch unaktiv ist. Das Wach-
stum dieser verpilzten Wurzel ist deshalb im ganzen nicht gut.

2) Wurzelbau der endotrophen Mykorrhiza.

Bei fast allen Pflanzen von Ericaceen, die in den jungen Wurzel-
epidermiszellen Pilzfäden besitzen, sind die Epidermiszellen auffallend
vergrössert, während die Rinde und der Zentralzylinder sich schlecht
entwickeln. Die Epidermiszellen zeigen auch da, wo zahlreiche Pilz-

fäden vorhanden sind, kein Längenwachstum; sie werden grosse viereckige Zellen. Die Epidermiszellen der älteren Wurzeln werden aber
durch die Verdauung der Pilzfäden verkleinert und verschwinden später.

Die Rindenzellen mit Pilzfäden sind auch sehr vergrössert, dagegen
ist die Entwicklung des Zentralzylinders etwas schlecht. Die anderen
Teile zeigen aber normale Entwicklung.

ÄUSSERE GESTALT DER MYKORRHIZA.

Die Mykorrhiza stellt je nach der Verschiedenheit des Typus auch
verschiedene Verzweigung dar. Die Gestalt der Mykorrhiza ist becherähnlich oder elliptisch, deren Oberfläche gelbweiss und sammetähnlich ist. Bei der einfachen Mykorrhiza findet sich etwas regelmässige
Verzweigung, und die Wurzeln sind ziemlich verdickt. Die Oberfläche
der jungen Mykorrhiza ist weiss, wird aber später gelb-braun. Der
Typus I. 2. B, der von einer festen Pilzschicht umhüllt ist, zeigt
ein ganz ähnliches Aussehen wie der normale. Die endotrophe Mykorrhiza, besonders die des Typus III. 1. ist haarartig und verzweigt
sich wiederholt. Dagegen findet bei dem Typus III. 2. nur verhältnismässig geringe Verzweigung statt. Die Mykorrhiza der Gramineen
gehört auch zu diesem Typus, aber sie verzweigt sich stark und
unregelmässig und lässt sich dadurch von den normalen Wurzeln
deutlich unterscheiden

Alle Mykorrhizien verzweigen sich im allgemeinen stark, aber ihre
Entwicklung ist schlechter als die der normalen Wurzeln, obwohl ein
anormales Dickenwachstum bei einigen Mykorrhizien häufig zu erkennen ist.

ZUSAMMENPASSUNG

1) Die meisten Gewächse im Solfataren-Gebiete auf dem Berg
Hakkoda besitzen Mykorrhiza, die zu verschiedenen Typen gehört.

2) Die äussere Form und der innere Bau der Mykorrhiza im
Solfataren-Gebiete sind ganz ähnlich wie die der auf gewöhnlichen
Waldböden gefundenen.

3) Die anormale Verdickung gewisser ektotrophen Mykorrhiza
wird nicht nur vom Pilzmantel, sondern auch vom radialen Wachstum
der Epidermiszellen hervorgerufen.

Report of the Biological Survey of Mutsu Bay.

17. Hirudinea.[1]

By

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(With 3 text-figures).

The fauna of Mutsu Bay can not be said to be rich in Hirudinea, as it includes so far only three species of this group of Annelids. Two of these are parasitic on teleostean Fishes, while the third was found upon a Mollusc, a case quite exceptional among the Ichthyobdellidae. The absence of the genus *Pontobdella*, by no means rare on our coasts, is rather conspicuous.

The three leeches now known to occur in Mutsu Bay, systematically arranged, are as follows:

Order Rhynchobdellida
Family Ichthyobdellidae

Gen. *Ichthyobdella* DE BLAINVILLE 1827, emend. OKA 1910.
  1. *I. uobir* OKA 1910.
Gen. *Callobdella* VAN BENEDEN et HESSE 1864, emend. OKA 1910.
  2. *C. livanovi* OKA 1910.
Gen. *Ostreobdella* OKA 1927.
  3. *O. kakibir* OKA 1927.

It must be remarked that the classification of marine leeches is still in a primitive condition, being based almost exclusively upon external characters, such as form, colour, annulation, size of suckers, etc. It is therefore evident that, when the internal organization of this group is better known, a large part of the species would have to be rearranged, and the generic designations now in use are to be considered, so far as such forms are concerned, as provisional.

1. Ichthyobdella uobir OKA 1910.

OKA, A. Synopsis der japanischen Hirudineen, mit Diagnosen der neuen Species. Annot. Zool. Japon., Vol. VII. 1910.

The body is elongated, depressed, with rounded margins, and divided into two regions, the neck and body, the posterior fourth of the former being slightly narrowed to form the clitellum. The anterior sucker is cup-shaped, not much wider than the neck, and is directed ventrally. The posterior sucker is hemispherical with a diameter about equal to the widest part of the abdomen, and is fixed to the latter by a rather narrow peduncle. Specimens kept in alcohol for a long time are of a uniform pale greyish colour, but in fresh state the dorsal surface presents a series of indistinct transverse bands of a

A

B

C
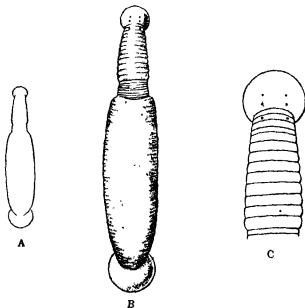
Fig. 1. *Ichthyobdella uobir.*

A   Outline of body, natural size.
B   Entire animal,   ×2
C   Anterior region,   ×4

brownish tint, that on the clitellum being somewhat darker than the rest. Besides, the oral sucker is ornamented with a narrow crescentic

band of the same colour about half way between its margin and the centre.

The largest specimen measures about 36 mm. in length and 7 mm. in breadth at the widest part, which lies in the middle of the abdomen.

The annulation is on the whole rather indistinct, especially in the abdominal region, where the integument appears in may specimens only irregularly wrinkled. In reality it consists for the most part of triannulate somites, whose primary rings are more or less clearly subdivided into secondary annuli. In the neck the rings are more distinct, and thirteen or fourteen of them may be counted in front of the clitellum. The latter is composed of seven or eight rings, much narrower than those of the neck proper. The anterior sucker shows on the dorsal surface indications of annulation, but too faint to be exactly counted. No such trace is visible on the posterior sucker.

The anterior sucker is circular in outline, and attached excentrally behind the centre. The mouth opening is very small, and is situated nearly at the centre of the concavity.

There are three pairs of eyes, two on the dorsal surface of the anterior sucker and one on the dorsal surface of the anterior part of the neck. They are small, black, and arranged as shown in Fig. 1, C.

The sexual pores are both on the ventral surface of the clitellum. The male orifice, which occupies the median region, is very large and conspicuous. The female pore, on the contrary, is extremely small and often difficult to detect ; it is located near the posterior margin of the clitellum.

The anus is separated from the posterior sucker by two postanal annuli.

Six pairs of low round elevations which are sometimes observable on the ventral surface of the abdomen indicate the positions of the testes.

Locality : Higashi Tazawa-oki ; on the inner surface of operculum of *Gadus macrocephalus.* 8. II. 1929. Twelve specimens.

2. Callobdella livanovi OKA 1910.

OKA, A. Synopsis der japanischen Hirudineen, mit Diagnosen der neuen Species. Annot. Zool. Japon., Vol. VII. 1910,

The body is cylindrical or claviform, little if at all depressed, and terminated by a sucker at each end. The length is about 30 mm. and the breadth about 4 mm. at the widest part. The body proper is divided into two regions, as in the preceding species, but unlike the latter the abdomen is provided with twelve pairs of globular respiratory vesicles, some of which, however, may be hidden when contracted. The anterior and posterior suckers are both separated from the body by well marked constrictions.

The anterior sucker is hemispherical or discoidal, about as long as wide, and fixed excentrically. It presents on its dorsal surface a number of shallow concentric furrows, which are no doubt interannular
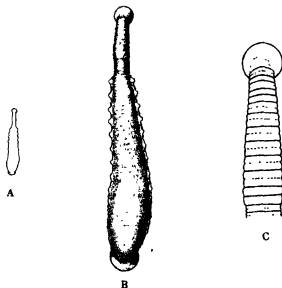
Fig. 2. *Callobdella livanovi.*
A Outline of body, natural size.
B Entire animal, ×4
C Anterior region, ×10

furrows of the cephalic region. The mouth opening is at the centre of the lower surface of the sucker. There are no eyes.

The neck is formed of about twenty rings of unequal size, the larger ones being subdivided by a more or less distinct transverse furrow. The posterior third of the neck is transformed into a clitellum, on the ventral surface of which the sexual pores are placed. The latter are separated from each other by four small rings, and between the female pore and the commencement of the abdomen are two small rings, of which the posterior is generally concealed in the deep furrow separating the cervical region from the abdominal.

In the abdominal region it is easy to recognize from the position of the lateral respiratory vesicles, that each somite consists of six secondary rings resulting from the subdivision of the three primary annuli. There are twelve such somites, then follow six rings forming three groups of two rings each. The anus opens on the last ring but one, at some distance from the posterior sucker.

The posterior sucker is relatively small and directed posteriorly, so that it looks like a simple continuation of the abdominal region. Like the anterior sucker it presents a number of parallel circular furrows, indicating the boundaries of the postabdominal annuli, but they become too faint posteriorly to be counted with certainty.

This species is remarkable for its extreme variability in coloration. The ground colour is in some specimens dark olive green, almost black, in others reddish brown, while in still others it is pale brown or even whitish. In certain individuals the dorsal and ventral surfaces are of different colours, for instance, the ventral surface being pale brown and the dorsal blackish. The respiratory vesicles are always opaque white, and may be joined with one another by a narrow longitudinal white line running along the lateral margin of the body. In addition to this there is a longitudinal series of small white spots arranged metamerically at a little distance from the lateral vesicles and on the same ring, both dorsally and ventrally. These spots as well as the marginal lines are of course not so conspicuous on light coloured individuals as on dark ones.

Localities:

Horotsuki, 18. V. 1927. 2 specimens.

    „   , on *Tetrodon* sp. 20. V. 1927. 2 specimens.

Asamushi, 23. VI. 1927. 1 specimen.

    „   , on *Cottus* sp. 7. VII. 1927. 9 specimens.

Asamushi, on *Sebastichthys* sp. 29. VII. 1927. 5 specimens.
    ,,   , on *Sebastodes schlegeli*. 29. VII. 1927. 8 specimens.
    ,,   ,   ,,   ,,   5. VIII. 1927. 27 specimens.
    ,,   ,   ,,   ,,   11. VIII. 1927. 10 specimens.
    ,,   , on *Sebastichthys* sp. 14. VIII. 7 specimens.
    ,,   , on *Sebastodes mitsukurii*. 16. VIII. 1927. 8 specimens.
    ,,   ,   ,,   ,,   26. VIII. 1927. 2 specimens.
    ,,   ,   ,,   ,,   15. IX. 1927. 21 specimens.

As may be seen from the above list, this is by far the most common of the marine leeches in Mutsu Bay. It is found almost exclusively upon Acanthopterygian fishes.

3. Ostreobdella kakibir OKA 1927.

OKA, A. Sur une nouvelle Ichthyobdelle parasite de l'Huitre Proc. Imp. Acad, Vol. III 1927.

This is a very small leech, the largest specimen measuring only
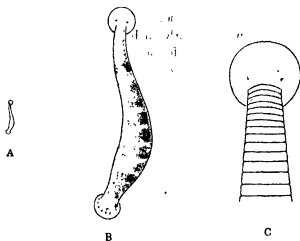
Fig. 3. *Ostreobdella kakibir*
A Outline of body, natural size.
B Entire animal. ×6
C Anterior region, ×15

14 mm. in length and 2 mm. in breadth at the widest part. The body is claviform or fusiform, tapering toward both ends, where it bears

a relatively large sucker. Unlike most of the Ichthyobdellidae, there is no distinction between the neck and abdomen, and the cross section is circular at any point of the body. The clitellar region, too, cannot be distinguished by its form externally.

The surface of the body is perfectly smooth, no papillae or tubercles being recognizable. The colour in general is a dark brown, a little paler on the ventral surface. An oval whitish patch on the dorsal surface indicates the position of the clitellar region. Behind this the ground colour is interrupted metamerically by transverse bands of whitish tint, which may be joined with one another by narrow longitudinal streaks of the same colour. In the cervical region, the transverse bands are broken up into transverse rows of round patches, which may be jointed in the same way by longitudinal lines.

The anterior sucker is circular and disc shaped; it is attached to the neck excentrically posterior to the centre. The mouth opening is situated at the centre of the under surface. The upper surface is convex and shows a number of more or less distinct concentric furrows, evidently the interannular furrows of the cephalic region.

A pair of small black eyes are seen on the dorsal surface of the anterior sucker. They are rather wide apart, and are separated from the neck by two cephalic rings. In specimens preserved in alcohol they are generally concealed by the brown pigment of the skin.

The number of rings composing the body proper is about 80. In general four rings form a somite, of which the second is easily recognizable by the presence of the whitish transverse band referred to above.

This species is very remarkable in having the rings subdivided by delicate annulations into a definite number of minute annuli. Toward both extremities, however, there is no trace of such secondary annulation.

The sexual pores are situated on the ventral surface of the clitellar region about opposite to the large white patch mentioned above. They are separated from each other by three rings.

The anus is placed on the dorso-median line just behind the last ring, i. e. between the abdomen and posterior sucker.

The posterior sucker is circular, cup-shaped, and directed obliquely backward. It is divided into rings by interannular furrows, of which the first two or three are fairly distinct, while the succeeding ones are

in most cases too faint to be counted   Eleven black eye-like spots are arranged along the posterior and lateral margins of the sucker

Locality   Asamushi, on the shell of living *Ostrea gigas* 24 III 1927   Two lots, 7 and 2 specimens

Studies on the Hepaticae of Japan. III.

By

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(With Pls XXI-XXIII & 13 Text-Figures)

## Genus: **WIESNERELLA** SCHIFFNER (1896)

Wiesnerella denudata (MITT.) STEPHANI

(Text-Figs 1-2)

Dumortiera denudata
MITTEN, Proc Linn Soc
Vol V, p 125 (1860)
Wiesnerella denudata
STEPHANI, Spec Hepat
Vol I, p 154 (1899) &
Vol VI, p 18 (1917)

Monoicous or di-
oicous. Hygrophyte
Growing in extended,
dark to light green,
shiny layers. Thallus
0.8-1.2 cm wide and
6-8 cm. long firm
and leathery, furcate
but apparently mono-
podial at lower parts,
margin repand-undu-
late, obcordate at the
apex. Dorsal epider-
mis composed of 4-
7-angled cells with
thin walls, mostly
30-45 μ wide (averag-
ing about 38 μ) and
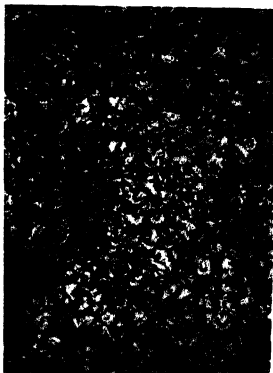
Text Fig 1 Growing habit of *Wiesenerella denudata* with ♀ & ☿ receptacles. ca 1/2
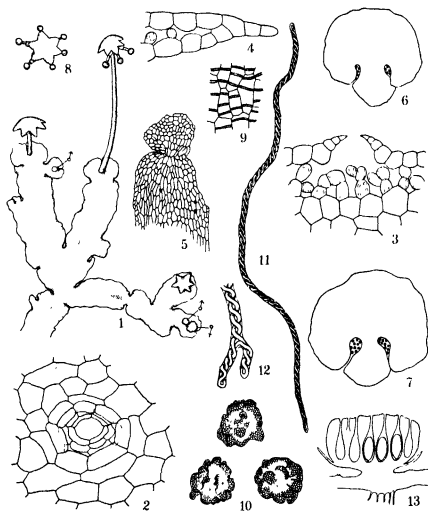
Text Fig 2   *Wiesnerella denudata* (MITT) STEPHANI

1 Plant bearing ♀ & ♂ receptacles, in nat. size.   2 Pore, in surface view,
×160   3 Ditto, in cross-section, ×160   4 Marginal portion of thallus, in cross-
section, ×160. 5. Ventral scale with appendage, ×42   6 Cross-section of female
peduncle, cut near apex, ×26   7. Ditto, near base, ×26.   8 Upper surface view
of female receptacle, in nat. size   9   Capsule-wall, in surface view, ×160.   10.
Spores, ×280   11 Elater, ×280.   12 End of branching elater, ×320.   13
Longitudinal section of ♂ receptacle, ×14

48–93 μ long (averaging about 70 μ). Pores (with their surrounding
cells) simple, elevated, mostly 90–105 μ wide and 117–123 μ long,
with 3–4 concentric rings, each ring being usually composed of 4
hyaline cells. Rhizoids numerous and almost colourless, smooth or
tuberculate. Air-chambers low, their boundaries distinct, occupying
about 1/8 of thickness in the middle and almost all of it in the marginal
portion. Midrib slightly prominent below; compact ventral tissue mostly
11–15 cells thick in the median portion, the walls thin throughout,
oil-cells distinct and scattered, gradually passing into the lamina, ending
in a 1-celled margin. Ventral surface green, scales in one row on
each side of the midrib, hyaline and very tender, broadly lunate with
a rotundate appendage and a few oil-cells. ♀ receptacle convex and
hemispherical, light green, bearing simple pores on upper surface,
divided to the middle into 5–7 (normally 6) acutely triangular lobes.
Capsule subglobose, rather longly pedicellate, exserted from the in-
volucre, irregularly dehiscing by four valves; walls of a single layer
of cells, with yellowish annular thickenings. Peduncle of ♀ receptacle
25–35 mm. long, hyaline except purple base, with 2 rhizoid-furrows.
Spores 40–48 μ in diameter, blackish-brown, covered with very un-
dulating folds, minutely reticulate. Elaters brown, slender and bent,
8–10 μ broad and 306–415 μ long, bispiral, longly attenuate, very
rarely branched. ♂ receptacle disciform, 1–1.5 mm. in diameter,
arising from the apex of a branch, the peduncle very short and broad.

 • *Fr.* April — May.

 Hab. On wet soil or rocks in the mountainous regions.

 Loc.

 Honshiu: Yugashima, prov. Idsu (C. Tsuge, 1886); Insl. Miya-
jima, prov. Aki (Y. Horikawa, no. 74, May 1923); Iwakuni, prov.
Suwo (Y. Horikawa, no. 281, March 1927).

 Shikoku: Mt. Washio, prov. Tosa (Y. Horikawa, no. 160,
March 1924).

 Kiushiu: Okawamura, Tamana-gôri, prov. Higo (Y. Horikawa,
no. 415, Apr. 1927); Watari, prov. Higo (K. Mayebara, no. 47,
Apr. 1929); Mt. Aoidake, prov. Hiuga (Y. Horikawa, no. 407, 433
& 475, Apr. 1927); Kagoshima, prov. Satsuma (Y. Horikawa, no
475, Apr. 1927).

 Formosa: Mt. Sedopan, Taigo-gun, prov Shinchiku (Y. Shimada,

no. 27, June 1928).

Distr. Himalaya and Hawai Isl.

Remarks. About ten years before the appearance of the genus *Wiesenerella* by SCHIFFNER, the late TSUGE * already figured this plant as *Reboulia* sp. basing on fertile specimen collected by himself at Yugashima in the province of Idsu.

Genus: MARCHANTIA LINNÉ (1753)

Marchantia diptera MONTAGNE
(Pl. XXI & Text-Fig. 3)

Marchantia diptera MONTAGNE, Ann. Sc Nat, p 243 (1843).
Marchantia calcarata STEPHANI, Bull Herb Boiss Vol. V, p 98 (1897).
Marchantia planispora STEPHANI, Spec Hepat Vol I, p 170 (1899)

Dioicous Mesophytic or somewhat hygrophytic. Growing in extended dense layers Thallus pale to deep green, often glaucous, usually pigmented with purple, especially near the margin and on the lower surface, mostly 0.6–1.1 cm wide' and 3–5 cm. long, repeatedly dichotomous, texture robust and leathery, margin more or less crispate but entire. Dorsal epidermis composed of 4–7-angled cells with thickened walls, sometimes in two layers, mostly 24–45 μ wide (averaging about 34 μ) and 63–75 μ long (averaging about 69 μ), papillae absent. Pores (with their surrounding 'cells) mostly 72–95 μ wide and 100–117 μ long, surrounded usually by six rows of cells (3 in the upper and 3 in the lower series), inner opening usually cruciate (often 5- or 6-sided) the bounding cells with resinous deposits. Air-chambers present everywhere, their boundaries distinct when viewed through the epidermis, occupying ca. 1/8 of thickness in the middle. Midrib prominent below; compact ventral tissue mostly 22–27 cells thick in the median portion, the walls pigmented with purple and infected with fungus, sclerotic cells usually distinct, scattered, mostly 9–14 in a cross-section, confined to median portion. Ventral scales purple, in two rows on each side of the midrib, the laminar scales alternating with the median ones and only a little nearer to the margin, appendages of median scales ovate-orbicular, mostly 0.5–0.67 mm. long and

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* TSUGE, C., Hepat, of Hakone, Idsu, Nikko, Tokyo and its vicinity. MSS. It is preserved at Bot. Institute, Tokyo Imp. University.

wide, apex rounded to broadly obtuse, margin entire or vaguely
dentate, cells showing a gradual decrease in size towards the margin,
cells containing oil-bodies present. ♀ receptacle disciform, 7-13 mm.
broad (mostly 10 mm.), usually not deeply 9-lobed (often 8-, more
rarely 7-, 10-, 11- or 6-lobed), the lobes when young or sterile
bended downwards, when fertilized becoming horizontal, 1.5-2 mm.
long, flat, dilated at the truncate to emarginate apex, basal sinus
considerably broader than the others, two basal lobes sometimes far
larger than the others, disc with a median papilliform protuberance
(about 1 mm. in diameter) and corresponding distinct ridges with the
lobes. Capsule rather longly pedicellate, somewhat exserted, oval and
brownish yellow. Peduncle of ♀ receptacle 3-7 cm. long (mostly
5 cm.), with 2 rhizoid-furrows and a single broad dorsal band of air-
chambers, hairs scattered and rather numerous. Involucre usually
pigmented with purple, deeply and irregularly lobed, the lobes longly
acuminate. Spores brownish-yellow, 20-24 μ in diameter, coarsely
mammillose. Elaters yellow, 6-8 μ wide and 288-750 μ long, bispiral,
longly attenuate to slender extremities, rarely shortened or branched.
♂ receptacle mostly 5-7 mm. wide, disciform, shortly lobed, the lobes
mostly 8 (rarely 7), rounded and with thin, revolute wavy margin,
ventral scales restricted to the middle portion of disc; peduncle 10-
14 mm. long, usually with 2 to 4 rhizoid-furrows, destitute of dorsal
air-chambers, nearly smooth. Cupules deeply lobed, the lobes acute,
dentate to short-spinose on the sides, outer surface with epidermal
papillae.

    Fr. June.

    Hab. On moist soil among rocks and on banks.

    Loc.

    Honshiu; Mt. Komagadake, prov. Ugo (I. KASHIMURA, no. 26,
July 1928); Obara-mura, prov. Rikuzen (Y. HORIKAWA, no. 1020,
Oct. 1927); Mt. Temmoku, prov. Kai (K. TAMURA, no. 70 a, Oct.
1902); Mt. Amagi, prov. Idsu (K. HISAUTI, no. 1, March 1928);
Takahashi-machi, prov. Bitchu (M. INUMARU, no. 1, Nov. 1928); Hiro-
shima, prov. Aki (Y. HORIKAWA, no. 94, May 1923 & no. 208, May
1924); Iwakuni, prov. Suou (Y. HORIKAWA, no. 282, March 1927).

    Shikoku: Kôchi-park, prov. Tosa (B. KUZUME, no. 223, Aug.
1926); Mt. Ishiduzi, prov. Iyo (I. KASHIMURA, June 1930).

Kiushiu: Nankwan-machi, prov. Higo (Y. HORIKAWA, no. 640, Apr. 1927); Hieda, Kumamoto, prov. Higo (Y. HORIKAWA, no. 634, Apr. 1927); Aida, prov. Higo (K. MAYEBARA, no. 5, Feb. 1927 & no. 16, 33, 36, March 1927); Nishize, prov. Higo (K. MAYEBARA, no. 37, March 1927 & no. 41, Apr. 1928); Watari, prov. Higo (K. MAYEBARA, no. 44, 45, Apr. 1928); Mt. Aoidake, prov. Hiuga (Y. HORIKAWA, no. 447, Apr. 1927); Aoshima-mura, prov. Hiuga (Y. HORIKAWA, no. 362, Apr. 1927); Ishûin-machi, prov. Satsuma (A. IWAKAWA, no. 4, Feb. 1929); Kagoshima, prov. Satsuma (Y. HORIKAWA, no. 565, 581, 582, 583, Apr. 1927).

Formosa: Mt. Sumahan, Taiko-gun, prov. Shinchiku (Y. SHIMADA, no. 24, June 1928).

Distr. The species endemic?

Remarks. Throughout the southern part of Japan this species is exceedingly abundant. Towards the north *Marchantia polymorpha* makes its abundance and *Marchantia diptera* becomes less and less at last quite extinct. This belongs to an exceedingly variable species according to the differences in environmental conditions. Fertile and sterile female receptacles are apparently so distinct from each other but they are connected by intermediate ones (Text-Fig. 3), when ♀

Text-Fig. 3. Some types of imperfect female receptacle, ca. ×2.

receptacle is young or fertilization has not yet taken place, the lobes do not spread horizontally but extended downwards and under this circumstance, the median protuberance and the radiating ridges are only slightly developed. The number of lobes in ♀ receptacle varies rather widely. Following table is an example counted from 282 numbers.

TABLE 1. Lobe-number of ♀ receptacles.

Nos of lobes	6	7	8	9	10	11	6 groups
Frequency (f)	1	7	37	232	3	2	282
Percentage	0.76	2.48	13.12	82.27	1.07	0.71	100

Taxonomically *Marchantia diptera* has a deep resemblance to *M. paleacea* BERT., * but it is provided with epidermal papillae on the outer surface of cupules.

Marchantia radiata HORIKAWA, sp. nov.

(Pl. XXII Text-Fig. 4)

Dioicous. Mesophytic or somewhat hygrophytic. Growing in extended dense layers, green to dark green, more or less shiny. Thallus repeatedly dichotomous, mostly 4–6 mm. broad and to 3 cm. long, firm and leathery, sometimes pigmented with purple on ventral surface, margin nearly entire. Dorsal epidermis composed of 5–7-angled cells with slightly thickened walls, sometimes in 2 layers, mostly 45–64 μ long (averaging about 55 μ) and 23–30 μ wide (averaging about 26 μ), papillae absent. Pores (with their surrounding cells) mostly 90–120 μ long and 78–90 μ wide, surrounded usually by 6–8 rows of cells, inner opening usually 4-sided, rarely 3- or 5-sided, not cruciate, each bounding cell often projecting inwards in the form of a rounded papilla. Air-chambers present everywhere, their boundaries distinct when viewed through the epidermis, occupying ca. 1–5 of thickness in the middle. Midrib prominent below; compact ventral tissue mostly 21–30 cells thick in the median portion, the walls pigmented with purple and more or less thickened, infected with fungus, sclerotic cells distinct, scattered, mostly 20–30 or more in a cross-section, usually confined to median region. Ventral scales pigmented with purple, in two rows on each side of the midrib, the row of laminar scales more or less irregular but tending to alternate with the median scales and not much nearer the margin, appendages of median scales ovate in outline, mostly 0.23–0.27 mm. wide, 0.31–0.45 mm. long, apex usually apiculate or acute, margin irregularly toothed, the teeth mostly one-celled, cells showing a gradual and slight decrease in size towards the margin, cells containing oil-bodies nearly always lacking. ♀ receptacle circular in outline, 6–7 mm. in diameter, usually deeply and radialsymmetrically 11-lobed (often 10-, rarely 9-, 12-lobed, the lobes spreading at maturity, ±2 mm. long, suddenly dilated at the

* MÜLLER, K., RABENHORST's Kryptogamen-Flora, 2 Aufl. 6 Bd. I Abt., s. 307 (1907);
EVANS, A., Trans. Connecticut Acad. Arts and Sci., Vol. 21, p. 253 (1917).

truncate to emarginate apex, upper surface of disc and lobes usually convex, the median papilliform protuberance distinct sometimes indistinct, basal sinus as same broad as the others. Capsule rather longly pedicellate, slightly exserted, oval and yellow. Peduncle of ♀ receptacle 20–35 mm. long (mostly 30 mm.), with 2 rhizoid-furrows and a single broad dorsal band of air-chambers, hairs scattered and rather numerous, more crowded at the upper end. Involucre hyaline, folded and revoluted, the lobes rounded and entire. Spores yellow, 23–27.7 μ in diameter, bearing a series of very low and narrower ridges not forming a network. Elaters yellowish-brown, mostly 6–8 μ wide, 472–540 μ long, bispiral. ♂ receptacle mostly 6–8 mm. or more broad, palmately 4–9-lobed, the basal sinus a very broad angle or a straight line, the lobes mostly 3–5 mm. long, 1.5–2 mm. wide, rounded at the apex, with thin somewhat revolute margin extending across the basal sinus, ventral scales imbricated, mostly in two rows; peduncle 15–23 mm. long, with 2 to 4 rhizoid-furrows, destitute of dorsal air-chambers, hairs scattered throughout. Cupules irregularly denticulate to ciliate, the teeth mostly 2–6 cells long, epidermal papillae lacking.

    Fr. June.

    Hab. On rocks and on soil among rocks in rather moist places.

    Loc.

    Honshiu: Mt. Temmoku, prov. Kai (K. TAMURA, Oct. 1912); Hiroshima, prov. Aki (Y. HORIKAWA, no. 202, Apr. 1924; no. 1819, June 1930); Yahata-mura, prov. Aki (Y. HORIKAWA, no. 212, May 1924); Iwakuni, prov. Suou (S. ONO, June 1929).

    Shikoku: Prov. Tosa (T. YOSHINAGA, June 1930); Kôchi-park, prov. Tosa (Y. HORIKAWA, no. 152, March 1924).

    Kiushiu: Nankwan-machi, prov. Higo (Y. HORIKAWA, no. 219, July 1925); Sakaki-mura, prov. Higo (Y. HORIKAWA, no. 313, Apr. 1927); Kumamoto, prov. Higo (Y. HORIKAWA, no. 217 & 218, March 1925, no. 632, Apr. 1927); Aida, prov. Higo (K. MAYEBARA, no. 42 & 43, Apr. 1928); Watari, prov. Higo (K. MAYEBARA, no. 46, Apr. 1928); Mt. Aoidake, prov. Hiuga (Y. HORIKAWA, no. 478, Apr. 1927); Aoshima-mura, prov. Hiuga (Y. HORIKAWA, no. 365, Apr. 1927); Kagoshima, prov. Satsuma (Y. HORIKAWA, no. 543, 544, 545, 546, 575, 576, 577, 578, 579, 580, 584, 611, Apr. 1927).

    Formosa: Mt. Sumahan, Taiko-gun, prov. Shinchiku (Y. SHIMADA,

no. 25. June 1928) ; Kômôkwan, Chikunan-gun, prov. Shinchiku (Y. SHIMADA, no. 2, Nov. 1927).

Distr. The species endemic.

Remarks. The present species is nearly related to *Marchantia emarginata* NEES.(=*M. palmata* NEES.), * but the latter differs from the former in the occurence of epidermal papillae and the semi-circular disc of ♀ receptacle. The modifications in the lobe-number of ♀ and ♂ receptacle are as follows :

TABLE 2. Lobe-number of ♀ receptacle.

Nos. of lobes	7	8	9	10	11	12	13	7 groups
Frequency (f)	6	5	21	47	144	10	2	235
Percentage	2.53	2.10	8.94	20.90	61.29	4.27	0.87	100

TABLE 3. Lobe-number of ♂ receptacle.

Nos. of lobes	3	4	5	6	7	8	9	10	12	18	10 groups
Frequency (f)	1	31	54	93	54	28	11	2	1	1	276
Percentage	0.36	11.23	19.56	33.70	19.57	10.14	3.99	0.73	0.36	0.36	100
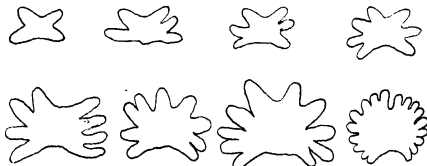
Text-Fig. 4. Some types of male receptacle, ca. ×2.

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* SCHIFFNER, V., Flore de Buitenzorg, 4 ème Partie, p. 31 (1900).

Marchantia tosana STEPHANI, Bull Herb Boiss.,
Vol V p 99 (1897)
(Pl XXIII

Dioicous Mesophytic or somewhat hygrophytic Growing in dense layers of a pale green to dark green colour Thallus repeatedly dichotomous, mostly 3–5 mm broad and to 3 cm long firm, sometimes pigmented with purple on ventral surface margin entire Dorsal epidermis composed of 4–8-angled cells with thin walls, of one layer, mostly 30–60 μ long (averaging about 43 μ) and 18–30 μ wide (averaging about 23 μ), papillae absent Pores (with their surrounding cells) mostly 130–216 μ long and 90–144 μ wide surrounded usually by six rows of cells (3 in the upper and 3 in the lower series) inner opening usually 4-sided not cruciate Air-chambers present everywhere, their boundaries distinct, occupying ca. 1/5 of thickness in the middle. Midrib prominent below compact ventral tissue mostly 18–20 cells thick in the median portion destitute of slime cells and sclerotic cells usually with slightly thickened walls, the walls pigmented with purple and infected with fungus Ventral scales purple in two rows on each side of the midrib median and laminar scarcely imbricated, the row of laminar scales not much nearer the margin, appendages of median scales ovate in outline, mostly 0 198–0.36 mm wide, 0 3–0 45 mm long, apex obtuse to apiculate, margin irregularly toothed, the teeth mostly one-celled, cells showing a gradual and slight decrease in size towards the margin, cells containing oil bodies lacking ♀ receptacle nearly semi-circular in outline, 4–6 mm wide usually deeply 5 7 lobed the lobes spreading at maturity, ± 1 1 mm long nearly flat the apex truncate to emarginate, basal sinus far broader (120°–180°) than the others, upper surface of disc and lobes usually plane Capsule longly pedicellate, exserted, oval and yellowish brown Peduncle of ♀ receptacle 15–25 mm long, with 2 rhizoid furrows and a single broad dorsal band of air-chambers, with a few scattered hairs or almost naked Involucre hyaline, somewhat irregularly lobed and crispate otherwise entire Spores brownish yellow, 24–30 μ in diameter, bearing low lamellae, forming a very distinct sometimes indistinct reticulum Elaters yellow, mostly 6 7.2 μ wide, 234–648 μ long, bi-spiral ♂ receptacle mostly 6–10 mm wide, palmately 4 8 lobed,

the basal sinus a very broad angle, the lobes veriable in size and
often transformed into thalli bearing cupules and rhizoids, with a thin
wavy margin extending across the basal sinus, ventral scales imbricated;
peduncle 12-15 mm long, with 2-4 rhizoid-furrows, destitute of dorsal
air-chambers, hairs scanty or almost naked. Cupules more or less
closely ciliate to denticulate, the cilia mostly one to five cells long,
outer surface without papillae.

   Fr. May — June

   Hab. On soil among rocks in rather moist places

   Loc.

   Shikoku : Kôchi, prov. Tosa (T. YOSHINAGA, June 1930).

   Distr. This species endemic.

   Remarks. Dr. OKAMURA * reported the regeneration of the ♂
receptacle in Japanese *Marchantia* (*M. cuneiloba* etc.)   The same
phenomenon is observed also often in *M. tosana* (for this material
the author acknowledges his thanks to Mr. T. YOSHINAGA) and *M
radiata* (cf. Pl. XXII, Figs. 21-24)   Surely it is caused by the
moisture in their habitats. The lobe-number of ♀ and ♂ receptacle
is shown in the following tables

<div align="center">TABLE 1.   Lobe-number of ♀ receptacle.</div>

Nos. of lobes	4	5	6	7	8	5 groups
Frequency (f)	2	20	17	36	1	76
Percentage	2.63	26.31	22.37	47.37	1.32	100

<div align="center">TABLE 5.   Lobe-number of ♂ receptacle.</div>

Nos. of lobes	4	5	6	7	8	5 groups
Frequency (f)	7	10	15	6	5	43
Percentage	16.28	23.26	34.88	13.95	11.63	100

* OKAMURA, S., Bot. Mag. Tokyo, Vol. 22 p 141 & p 177 (Japanese) (1908)

Genus: **MASTIGOPHORA** Nees (1838)

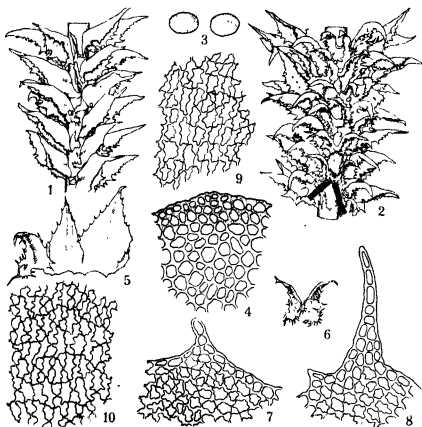### **Mastigophora spinosa** Horikawa, sp. nov.

(Text-Figs 5–6)

Sterile. Mesophyte. Plants attaining to 6 cm. in height, in golden-yellow dense tufts. Stems usually erect or procumbent, about 0 45 mm. in diameter, rigid and brittle, dark brown, yellowish near the apex, closely leaved elsewhere, slightly and irregularly, sometimes subfasci-culately branched, the branches lateral and patent, sometimes micro-phyllous. Rhizoids rather scarce, almost confined to the base of stem, fasciculate and colourless   Leaves imbricate, 1.77 mm. wide and

Text-Fig 5   *Mastigophora spinosa* Horikawa in nat size

1.33 mm. long, incubous, somewhat transversely inserted, semi-amplexi-caul, broader than long, deeply unequally divided into 3 patent seg-ments, the antical segment largest, oblong-ovate, usually with a small lobe near the antical base, the next segment similar to the antical segment, the most postical segment narrower, lanceolate, frequently with long spinous-teeth near the base, the apex arched downwards, all the segments being acuminate and strongly recurved and canali-culate, the margins somewhat distantly and irregularly spinous-dentate.

Cells of antical segment averaging 12μ at the margin, $14 \times 22 \mu$ in the middle and $14 \times 28 \mu$ at the base, stellate, trigones very large and confluent, pale yellow. Underleaves smaller than the leaves, mostly 0.67 mm. wide and 0.81 mm. long, erect-patent, the margins strongly recurved, canaliculate, usually deeply 2-lobed, lobes lanceolate, acuminate, the whole margin spinous-dentate, the spines at the base often curved.

Text-Fig. 6. *Mastigophora spinosa* HORIKAWA

1. Part of plant, antical view, ×12. 2. Ditto, postical view, ×12. 3. Cross-section of two stems, ×14. 4. A,portion of ditto, ×160. 5. Stem-leaf, dissected from the stem, ×14. 6. Underleaf, ×14. 7. A spine from antical margin of antical segment, ×280. 8. Two spines from postical margin of ditto, ×280. 9. Cells from middle of antical segment, ×280. 10. Cells from base of ditto, ×280

Hab. On the decaying logs in alpine district.

Loc.

Formosa: Mt. Arisan, Numano-daira (ca. 2500 m.), prov. Tainan
(A. NOGUCHI, Aug. 1928-type); Mt. Daibusan (ca. 3000 m.), prov.
Takao (Y. SHIMADA & S. ŌHASHI, Jan. 1928-cotype).

Distr. The species endemic.

Remarks. Among twenty species of the genus, the present species
seems to have a certain resemblance to *Mastigophora madagassa*
STEPH., which is known only from Madagascar *. The genus is new
to Japan.

Genus : RADULA DUMORTIER (1822)

Radula gigantea HORIKAWA, sp. nov.

(Text Fig. 7)

Dioicous. Xerophyte. Plants robust and large, usually about
4 cm. long and 2.5–3.6 mm. wide, growing in loose, entangled, olive-
brown to olive-green patches. Stems rather stout, 0.18–0.28 mm. in
diameter, reddish-brown, irregularly and loosely subpinnate, the branches
widely spreading, similar to the stem but often with smaller leaves.
Stem-leaves slightly subimbricate, horizontal, not decurved, the antical
lobe ovate in outline, longer than broad, about 1.8 mm. long and
1.4 mm. wide, attached by an almost longitudinal line of insertion,
somewhat rounded at the antical base and arching partially or wholly
across the axis, antical margin strongly rounded, the apex broadly
obtuse, postical margin also rounded, forming a almost straight line
with the keel, margin more or less wavy but entire throughout. Lobules
erect, longer than broad, 0.58–0.89 mm. long and 0.49–0.8 mm. wide,
4–8 times smaller than the lobes, nearly wholly or widely crossing
the stem, slightly inflated along the keel. Cells of lobe averaging
about 14–22 μ at the margin, 17–33 μ in the middle and 24 × 35 μ
at the base, the trigones large. Androecium occupying the whole,
less frequently only the apex or the base, of a lateral branch, bracts
mostly in 5–8 pairs, much smaller than the leaves, erect, closely
imbricate, unequally bilobed and ventricose.

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* STEPHANI, F., Spec. Hepat. Vol. IV, p. 40 (1909) & Vol. VI, p. 367 (1922).
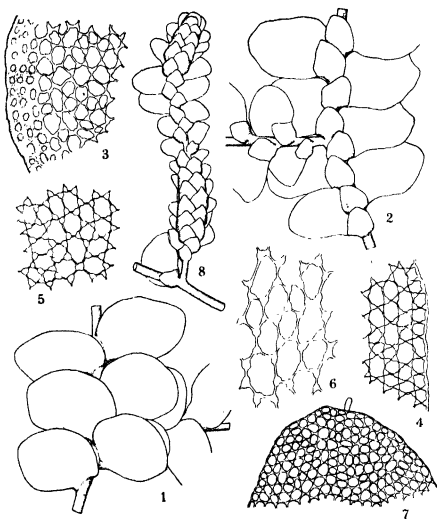
Text-Fig. 7. *Radula gigantea* HORIKAWA

1. Part of plant, antical view, ×16. 2. Ditto, postical view, ×16. 3. A portion of stem, in cross-section, ×144. 4. Cells from margin of lobe, ×233. 5. Cells from middle of ditto, ×233. 6. Cells from base of ditto, ×300. 7. Apex of lobule, ×160. 8. Androecium-bearing branch, ×9.

Hab. On rocks and bark of trees in the mountainous region.
Loc.
Honshiu: Mt. Madoyama, prov. Aki (A. NOGUCHI, no. 42, Oct.

1926); Insl. Miyajima, prov. Aki (Y. HORIKAWA, no. 36, Feb 1923-
cotype; no. 84, May 1923); Shiroyama, Iwakuni, prov. Suou (Y.
HORIKAWA, no. 285, March 1927).

Shikoku: Mt. Washio, prov. Tosa (Y. HORIKAWA, no. 170-type;
no. 171, March 1924).

Kiushiu: Kagoshima, prov. Satsuma (Y. HORIKAWA, no. 600,
602, Apr. 1927).

Distr. This species endemic.

Genus: **MADOTHECA** DUMORTIER (1922)

Madotheca ulophylla STEPHANI, Bull. Herb. Boiss.,
Vol. V, p. 97 (1897).
(Text-Fig. 8)

Dioicous. Xerophyte. Plants flaccid and fragile, large, about
4 cm. long and 3 mm. wide, growing in dense, entangled, dark green
patches. Stems flexuose, about 0.5 mm. in diameter, pale brown,
irregularly and longly branched, the branches usually bipinnate.
Stem-leaves imbricate, the lobes widely spreading and crossing the
stem, ovate in outline, about 2 mm. long and 1.7 mm. wide, the apex
rounded to broadly obtuse, the margin entire, most frequently remark-
ably crispate. Rhizoids from the base of underleaves, fasciculate and
brown. Cells of lobes averaging about 21μ at the margin, 30μ in
the middle and $30 \times 60 \mu$ at the base, the trigones rather smaller but
distinct. Lobules oblong-ovate, convex, mostly 0.85 mm. long and
0.45 mm. wide, narrowed at the obtuse apex, hardly decurrent, the
margin entire. Underleaves ovate-triangular, 0.8 mm. long and 0.7 mm.
wide, broader than the stem, approximate to slightly imbricate, the
apex obtuse to rounded, the margin frequently recurved, entire. ♀
inflorescence on short branches proceeding from the main stem or
branches, involucral bracts one pair, acute and slightly dentate; lobules
narrowly lanceolate, acuminate; bracteoles larger, dentate and acute.
Perianth broadly obovate, 2 mm. long and 1.6 mm. wide, the mouth
widely truncate, bilabiate, denticulate to ciliolate, pedicel hyaline and
rather long, 3 mm. long and 0.28 mm. wide. Capsule globose, brown,
about 0.8 mm. in diameter, irregularly longitudinally splitting into 6–8
valves. Spores nearly spherical, $30–45 \mu$ in diameter, greenish, finely
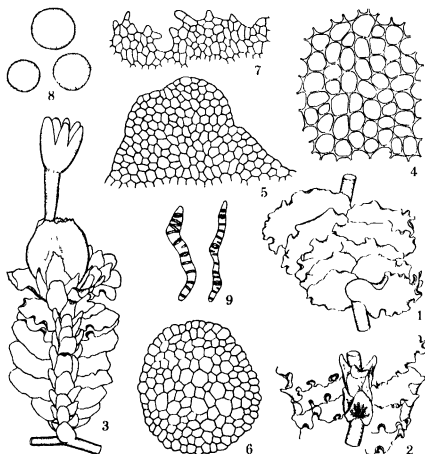
Text-Fig. 8   *Madotheca ulophylla* STEPHANI.

1. Part of plant, antical view, ×9.   2 Ditto, postical view, ×9.   3 Fertile
branch, postical view, ×9.   4. Cells from middle of lobe, ×160.   5 Apex of
lobule, ×113.   6. Cross-section of capsule-pedicel, ×113   7 Marginal part of
perianth, ×113.   8. Spores, surface view, ×280   9. Elaters, ×160

echinate-papillose. Elatèrs brown, variable in size, usually $12–18\,\mu$
wide, with irregular and incomplete spiral bands, branching one rarely
occurs.

    Fr.   March — April.

    Hab.   On barks and about the roots of trees.

    Loc.

Honshiu: The foot of Mt. Komagadake, prov. Shinano (Y. HORI-
KAWA, no. 119, July 1923); Mt. Fukuoji, prov. Aki (Y. HORIKAWA,
no. 24, Oct. 1922, no. 108, 109, June 1923); Yagi-mura, prov. Aki
(I. KASHIMURA, no. 8, March 1927); Ipsl. Miyajima, prov. Aki (Y.
HORIKAWA, no. 679, Apr. 1927); Iwakuni, prov. Suou (Y. HORIKAWA,
no. 277, 278 & 279, March 1927).

Shikoku: Mt. Yokogura, prov. Tosa (T. YOSHINAGA, no. 37, Apr.
1896); Ôdaru-yama, prov. Tosa (T. YOSHINAGA, no. 48, Apr. 1896).

Kiushiu: Kagoshima, prov. Satsuma (Y. HORIKAWA, no. 604, Apr.
1927).

Madotheca densifolia STEPHANI, Soc. Nat. Cherbourg, Vol. 29,
         p. 219 (1894) & Spec. Hepat. Vol. IV, p. 301 (1910).
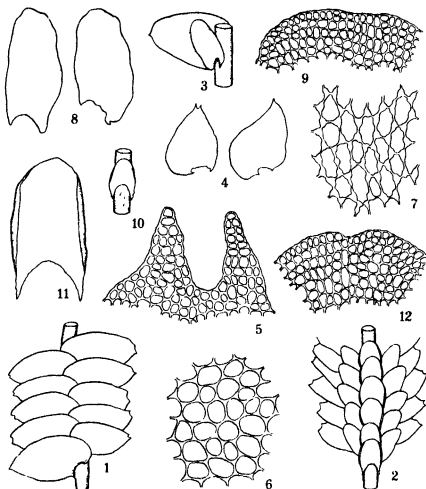
(Text-Fig. 9)

Sterile. Xerophyte. Plants large and robust, usually 8–10 cm.
long, 2–3 mm. broad, growing in loose, dark green to olive green
patches, slighly shiny. Stems rigid, about 0.5 mm. in diameter, red-
dish-brown, irregularly pinnate and more or less bipinnate, branches
usually of equal breadth throughout. Stem-leaves closely imbricate,
the lobes widely spreading and not or hardly crossing the stem,
slightly convex, ovate-oblong, mostly 2.2 mm. long, 1.5 mm. broad,
asymmetrical, narrowed towards the apex, which is acute to bidentate,
sometimes obtuse, the antical margin broadly rounded, entire, the
postical margin less arched, frequently with 1–2 teeth near the base.
Cells of lobe averaging about 15 μ at the margin, 22 μ in the middle
and 18 × 35 μ at the base, walls more or less thickened with distinct
trigones. Lobules oblong-ovate, mostly 1.2 mm. long, 0.6 mm. wide,
narrowed at the rotundate apex, hardly decurrent, the margin entire.
Underleaves nearly as broad as the lobules, imbricate, ovate-triangular,
the apex rounded or nearly truncate, the margin entire, frequently
narrowly recurved, longly decurrent and occasionally with teeth at
the base.

Hab. On moist rocks in sheltered places.

Loc.

Honshiu: Mt. Ôiwayama, Kamishinkawa-gôri, prov. Ettchû (K.
SHINNO, no. 10, Oct. 1929); Nagato-kyô, prov. Nagato (Y. HORIKAWA,

Text-Fig. 9. *Madotheca densifolia* STEPHANI.

1. Part of plant, antical view, ×9. 2. Ditto, postical view, ×9. 3. Lobe and lobule, postical view, ×9. 4. Lobes, ×9. 5. Apex of ditto, ×160. 6. Cells from middle of lobe, ×280. 7. Cells from base of ditto, ×280. 8. Lobules, ×26 9. Apex of lobule, ×160. 10. Underleaf, ×9. 11. Ditto, dissected from stem, ×26. 12. Apex of underleaf, ×160.

no. 293 & 294, Apr. 1927); Ditto (S. TAMURA, Jan. 1930).

Shikoku: Sugi, prov. Tosa (A. NOGUCHI, no. 118, March 1928).

Distr. China (Yunnan) and Himalaya (Kumaon).

Madotheca japonica SANDE-LACOSTE, Hep. Jav., p. 105 ;
STEPHANI, Spec. Hepat. Vol. IV, p. 313 (1910).
(Text-Fig. 10)

Dioicous. Mesophyte. Growing in flat and entangled, dark green
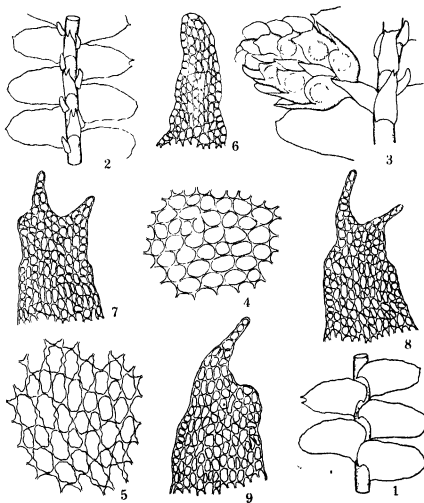
Text-Fig. 10. *Madotheca japonica* SANDE-LACOSTE·
1. Part of plant, antical view, ×14. 2. Ditto, postical view, ×14. 3. Ditto
bearing androecium, postical view, ×42. 4. Cells from middle of lobe, ×280.
5. Cells from base of lobe, ×280. 6. Upper half of lobule, ×160. 7, 8, 9. Upper
halves of underleaves, ×160.

patches. Stems usually about 6 cm. long, 0.22 mm. in diameter,
blackish, irregulary branched, the branches regularly pinnate. Stem-
leaves distant to approximate or slightly imbricate, oblong-oval, 1.5 mm.
long and 0.84 mm. wide, widely spreading, flat or slightly convex, the
antical margin broadly arched, not crossing the stem, the postical
margin less arched and usually slightly incurved, the apex acute,
bidentate or obtuse, margin nearly entire, sometimes slightly repand.
Cells of lobe averaging about 15 μ at the margin, 19 μ in the middle
and 18 × 42 μ at the base, middle lamella distinct, trigones and inter-
mediate thickenings distinct towards the basal region. Lobules minute,
narrowly ligulate to linear-oblong, about 0.18 mm. long and 0.11 mm.
wide, the apex usually obtuse, not decurrent at the base, flat or slightly
concave, margin entire throughout. Underleaves smaller, distant to
approximate, oblong-quadrate, 0.45 mm. long and 0.2 mm. wide, nearly
same broad as the stem, hardly decurrent, the apex usually bidentate,
margin entire. Androecia in short lateral branches, oblong-oval,
bracts in 2–4 pairs, closely imbricate, ventricose.

   Hab. On moist sheltered rocks.
   Loc.
   Shikoku: Mt. Yokogura, prov. Tosa (S. OKAMURA, March 1904);
Ditto (S. TAMURA, March 1930).
   Distr. This species endemic.

       Genus: LEJEUNEA LIBERT (1820) emend. EVANS (1900)

               Lejeunea aquatica HORIKAWA, sp. nov.
                         (Text-Fig. 11)

   Dioicous. Hygrophyte to hydrophyte. Plants growing in flat, pale
to yellowish-green mats. Stems prostrate, 15–25 mm. long and 0.13 mm.
in diameter, loosely adherent to the substratum, slightly and sub-
pinnately branched, the branches similar to the stem. Rhizoids scarce.
Leaves loosely imbricated, the lobes obliquely to widely spreading,
slightly convex, scarcely falcate, ovate-oblong, about 1.1 mm. long and
0.8 mm. wide, antical margin arching across or just beyond the axis,
rounded to broadly obtuse at the apex, the margin entire throughout.
Lobules very minute, frequently almost obsolete, about 0.1 mm. long
and 0.09 mm. wide, slightly inflated, subovoid, the free margin involute

except at the apex, with an 2-celled hyaline tooth at the free angle.
Cells of lobe almost plane, thin walled, trigones scarcely evident,
averaging 21 μ at the margin, 36 μ in the middle and 25 × 57 μ
at the base. Underleaves distant to contiguous, plane or nearly so,
orbicular, about 0.5 mm. long and 0.53 mm. wide, rounded and cordate
at the base, 1/3 bilobed, segments acute to obtuse, sinus acute to
obtuse, margin entire. Androecium occupying a short lateral branch;
bracts in 2–5 pairs, strongly inflated and with strongly arched keel,
subequally bilobed. Antheridia solitary, globose.
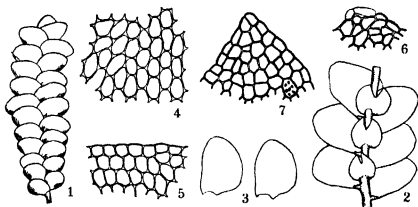
Text-Fig. 11. *Lejeunea aquatica* HORIKAWA.

1. Anterior part of plant, antical view, ×9. 2. Part of stem, postical view,
×14   3 Two lobes, ×14   4 Cells from middle of lobe, ×160. 5. Cells from
antical margin of lobe, ×160. 6. Apex of lobule, ×160. 7. Apex of a segment
from stem-underleaf, ×160

Hab. On wet rocks near waterfalls.

Loc.

Honshiu: Mitaki, a vicinity of Hiroshima, prov. Aki (Y. HORI-
KAWA, no. 1795-type, May 1930).

Distr. The species endemic.

Remarks. This species is closely allied to *Lejeunea Holtii* SPRUCE, *
a very rare species in Ireland but differs from it by the more im-
bricated and broader lobes.

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* SPRUCE, R., Journ. Bot. Vol. XXV, p. 33 (1887) & MACVICAR, S., Handb. Brit.
   Hep., p. 428 (1926).

Genus: EUOSMOLEJEUNEA SPRUCE (1885)

Euosmolejeunea auriculata STEPHANI, Spec. Hepat.,
Vol. V, p. 593 (1914).
(Text-Fig. 12)

Dioicous. Mesophyte to hygrophyte. Plants pale to glaucous-green, growing scattered among mosses. Stems prostrate, 4 cm. long, 0.18 mm. in diameter, sparingly and irregularly branched, the branches widely spreading. Rhizoids confined to the base of underleaves, short and scarce. Leaves contiguous to loosely imbricated, obliquely spreading, the lobes convex, ovate in outline, about 0.58 mm. long and 0.42 mm. wide, slightly revolute towards the apex, antical margin arching across
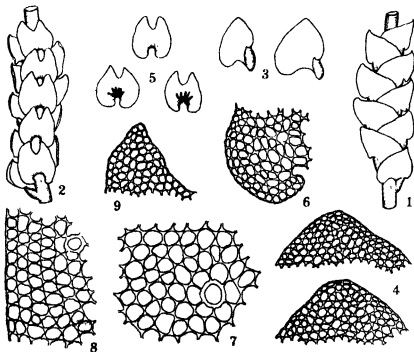
Text-Fig. 12. *Euosmolejeunea auriculata* STEPHANI.

1. Part of plant, antical view, ×26   2. Ditto, postical view, ×26   3. Stem-leaves, ×26.   4. Apices of lobes, ×160.   5. Stem-underleaves, ×26.   6 One half of basal portion of ditto, ×160.   7. Cells from middle of lobe, ×280.   8 Cells from margin of lobe, ×280.   9. Apex of one segment from stem-underleaf, ×160.

or just beyond the axis, rounded at the base, postical margin forming
a distinct but very obtuse angle with the keel, apex obtuse to subacute,
margin entire throughout. Lobules ovate-oblong in outline, mostly
0.26 mm. long and 0.1 mm. wide, inflated throughout, keel slightly
arched, free margin strongly involute to beyond the subacute apex,
then obliquely lunulate to the end of keel. Cells of lobe averaging
about 15 μ at the margin, 18 μ in the middle and 20 × 31 μ at the
base, with somewhat thickened walls and distinct triangular trigones,
frequently a few thick-walled cells scattered. Underleaves distant to
slightly imbricated, subappressed, nearly plane, orbicular, 0.46 mm.
long and 0.45 mm. wide, bifid about one third with obtuse or subacute
lobes separated by an obtuse sinus, deeply cordate at the base and
attached by a long, curved line of insertion, margins entire.

   Hab. Creeping over mosses in well sheltered places.

   Loc.

   Honshiu : Mt Mitaki, prov. Kai-type locality (K. TAMURA, no.
45, July 1903) ; Insl. Miyajima, prov. Aki (Y. HORIKAWA, no. 90,
May 1923) ; Nagatokyô, prov. Nagato (Y. HORIKAWA, no. 305, Apr.
1927) ; Ditto (A. NOGUCHI, no. 1383, Oct. 1928).

   Shikoku . Mt. Yokogura, prov. Tosa (Y. HORIKAWA, no. 192,
March 1924).

   Distr. This species endemic.

<div align="center">

Genus : **FRULLANIA** RADDI (1820)

Frullania viridis HORIKAWA, sp. nov.

(Text-Fig. 13)

</div>

   Dioicous. Xerophyte. Plants moderate in size, mostly 12–18 mm.
long and 1.8–2.05 mm. wide, light to yellowish green, growing in
depressed mats. Stems prostrate, rather closely adherent to the sub-
stratum, 0.12–0.17 mm. in diameter, yellowish-brown, greenish towards
the apex, usually bipinnate. Rhizoids fasciculate, reddish-brown and
rather numerous. Stem-leaves imbricate, the lobes widely spreading,
nearly ovate, mostly 1.25 mm. long and 0.85 mm. wide, somewhat
convex, rounded at the antical base and arching rather far across
the stem, the apex rounded to obtuse and not decurved, margin
somewhat repand but nearly entire. Cells of lobe containing numerous
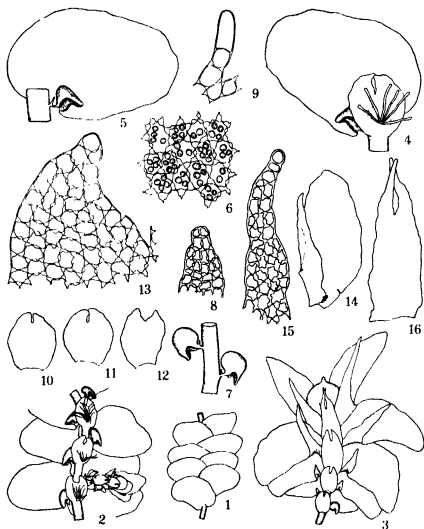
Text-Fig. 13. *Frullania viridis* HORIKAWA.

1. Part of stem, antical view, ×8. 2. Part of plant, postical view, ×16. 3. Fertile branch with young perianth, ×18. 4. Lobe, lobule and underleaf, postical view, ×38. 5. Lobe and lobule, postical view, ×38. 6. Cells from middle of lobe, ×233. 7. Lobules, postical view, ×42. 8, Ligulate lobule, ×160. 9. Stylus, ×233. 10, 11, 12. Stem-underleaves, ×42. 13. One segment of stem-underleaf, ×233. 14 Innermost bract of ♀ inflorescence, ×26. 15 Upper part of lobule of innermost bract, ×160 16. Innermost bracteole, ×42.

chloroplasts and oil-bodies, averaging about 23 μ at the margin, 26 × 30 μ
in the middle and 30 × 48 μ at the base, trigones very large and
somewhat confluent, intermediate thickenings usually distinct. Lobules
galeate, rarely ligulate, rounded vertically, usually smaller than the
underleaves, about to 360 μ in height and 306 μ broad, inflated,
parallel or subparallel with the stem and rather close to it. Stylus
minute and filiform, mostly 3-4 cells long and 1 or 2 cells wide at
the base. Stem-underleaves subimbricate to distant, large, about
thrice as broad as the stem, rotundate-oval, averaging about 540 μ
long and 450 μ wide, scarcely decurrent, 1/5 to 1/4-bilobed, the sinus
subacute to obtuse, lobes acute to subobtuse at the apex, margin
nearly entire. ♀ inflorescence borne on a short lateral branch. In-
volucral bracts 2-3 pairs, passing by insensible gradations into the
leaves, complicately and unequally bifid. Lobes of innermost bracts
ligulate-oblong, 1.5 mm. long and 0.66 mm. wide, obtuse at the apex,
margin slightly sinuate. Innermost bracteole free, lanceolate, 1 mm.
long and 0.33 mm. wide, bifid about 1/3 with a narrow sinus and
acute divisions, margin slightly sinuate. Lobule of innermost bracts
lanceolate, 1.2 mm. long and 0.25 mm. wide, acute at the apex, margin
slightly irregularly sinuate, usually bearing a cluster of short and
irregular cilia at the base.

    Hab. On the old trunk of tree fern (*Cyathea boninsimensis* CO-
PELAND).

    Loc.

    Bonin: Insl. Chichijima (T. SHIBATA, no. 259-type, Feb. 1927).

    Distrib. The species endemic.

EXPLANATION OF PLATES

PLATE XXI.

Marchantia diptera MONTAGNE

  Fig. 1. Female plant, in nat. size.

  Fig. 2. Male plant, in nat. size.

  Figs. 3, 4 & 5. Pores in cross-section. 3, 4, ×280. 5, ×160.

  Fig. 6. Inner openings of pores, ×160.

  Fig. 7, 8, 9 & 10. Ventral median scales with appendages, ×14.

  Figs. 11 & 12. Appendages of ditto, ×42.

PLATE XXII.

Marchantia radiata Horikawa

PLATE XXIII.

Marchantia tosana Stephani

Fig. 14.   Ditto, near apex, ×26
Fig. 15.   Part of involucre, ×14
Figs. 16, 17.   Ditto, ×113.
Fig. 18.   Spores, ×525.
Figs. 19, 20.   Marginal part of cupule, ×160.
Figs. 21, 22, 23 & 24.   Male receptacles, showing the lobes regenerate in thalli
       with cupules and rhizoids, in nat. size.
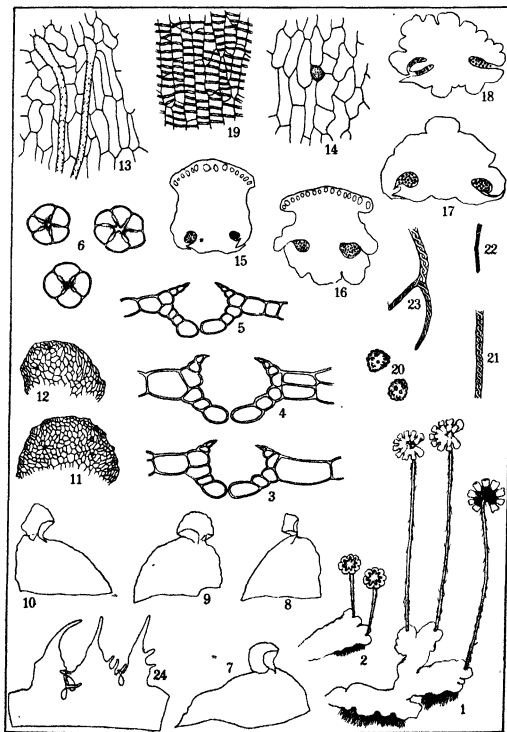
Author del.

Y. HORIKAWA: *Marchantia diptera* MONTAGNE.

Y. HORIKAWA: *Marchantia radiata* sp. nov.

Y. HORIKAWA: *Marchantia tosana* STEPHANI.

Note on *Pheretima agrestis* (Goto and Hatai), together with the description of four new species of the genus *Pheretima*.

By

Shinkishi Hatai.

Biological Institute, Tôhoku Imperial University, Sendai, Japan.

(With 8 Text-figures).

Perichaeta agrestis Goto and Hatai, Goto, S. and Hatai, S.· Ann. Zool. Japon.
    III, P. 17, 1898.
Pheretima agrestis (Goto and Hatai), Michaelsen, W.· Oligochaeta in Das Tier-
    reich, P. 272 and P 313. 1900.
Amyntas agrestis (Goto and Hatai), Beddard, F. E.· P. Z S., 1900
Pheretima agrestis (Goto and Hatai), Yamaguchi, H. Dôbutsugaku Zasshi
    (Zoological Magazine) Vol. 42, No. 496, P. 51, 1930.

In presenting *Pheretima agrestis* (Goto and Hatai), in his work
on "Oligochaeta", Michaelsen ('00) remarks that "Vielleicht eine
Varietat von *P. hilgendorfi*". In regard to this remark the present
writer only wishes to state that Dr. Michaelsen is mistaken in
uniting these two distinctly different species. *P. agrestis* possesses
three pairs of spermathecal pores in V/VI, VI/VII, VII/VIII instead
of the regular two pairs in VI/VII, VII/VIII of *P. hilgendorfi*. Al-
though both species possess a circular patch of light brown color, I
have definitely stated in the original paper that "A similar patch
or patches are present in *P. masatakae* Bed. and *P. hilgendorfi*
Mich., and in these species there are numerous small papillae on the
external surface of these patches, on which the so-called capsulogenous
glands open : but in the present species there are neither papillae on
the outside nor any capsulogenous glands on the inside. We think,
however, that these patches serve the same purpose as the genital
papillae."

Recently I had an opportunity to collect a number of *P. agrestis*
from various parts of Japan and was thus able to determine an extent
of variation. In order to avoid further confusion in regared to the
position of *P. agrestis* as an independent species and at the same
time to demonstrate the difference between this species and *P. hilgen-*

dorfi MICHAELSEN, I have made several illustrations which I am sure will clear the tangled situation. I only wish to point out that the majority of Japanese *Pheretima* possess more than one pair of intestinal coeca and also capsulogenous glands of various forms and in varied positions, and *Pheretima hilgendorfi* MICH. is not the only species favored by nature in these regards and, therefore, to include any *Pheretima* species with capsulogenous glands and also with several intestinal coeca in *P. hilgendorfi* is not always justified without paying due respect to other differences in character, together with other biological factors such as habitat, behavior, etc.

The original description of *P. agrestis* was published over thirty years ago and I shall recite and the whole statement for reference. "Length 100–160 mm., breadth 5.8 mm., number of segments 80–96. Clitellum XIV–XVI, without setae. Number of setae in the sperma-thecal region 36, more posteriorly 40 or so. Spermathecal pores three pairs, V/VI, VI/VII, VII/VIII. There are no genital papillae in this region: but there are two pairs of slightly elevated squarish patches of a light brown color inside the spermathecal pores, one in VII and the other in VIII. A similar patch or patches are present in *P. masatakae* BED. and *P. hilgendorfi* MICH., and in these species there are numerous small papillae on the external surface of the patches, on which the so-called "capsulogenous glands" open: but in the present species there are no papillae on the outside nor any capsulogenous glands on the inside. We think, however, that these patches serve the same purpose as the genital papillae. No male pores could be observed. First dorsal pore in XII/XIII.

Gizzard in VIII, IX: intestine beginning in XV, with one pair of coeca in XXVII bearing 7 pairs of secondary diverticula. These secondary diverticula are longest next the dorsum and thence gradually decrease in length towards the ventrum, the longest ones reaching as far anteriorly as segment XXIV. Thickened septa V/VI, VI/VII and X/XI, XIV, XV; septa VIII/IX, IX/X wanting. Spermathecae three pairs, in VI, VII, VIII, with diverticula longer than the main sac, but almost straight or very slightly winding (fig. 7). Sperm reservoir in XI, XII. Ovisac present. Prostate gland absent. Sperm ducts asymmetrical and terminating with bulbular swelling either in segment XIII or XVIII or even posteriorly.

Loc. Takahashi (Prov Bitchu), Tokorosawa (Prov. Musashi).
Oarai (Prov. Hidachi).

Among more than one hundred specimens of this species in our
hands we find some in which the first dorsal pore lies in XI/XII.
and a few without the modified patches above described, as also a
few with a pair of large papillae (of 0.8–1 mm. in diameter), in front
of the chaetal line in segment XVIII. All these variations, which
occur independently, were found in the specimens from "Oarai."

The number of localities where *P. agrestis* has been found are
now considerably increased · Sapporo, Hokkaido, wide distribution in
Aomori and Iwate prefectures, Sendai, Takahashi (Prov. Bitchu),
Tokorosawa (Prov. Musashi), Oarai (Prov. Hidachi), Oshima Island
(Prov. Kanagawa), Matsuyama (Prov Iyo), Tomitaka (Prov. Miyasaki)
and Kagoshima (Prov. Kagoshima)

For future reference I shall present data on the number of setae
in several segments of *P. agrestis* picked up at random from collec-
tions taken from various localities

Number of Setae on *P. agretis*.

No. of Segment Region	IV	VI	VIII	X	XVIII	XXX
Aomori	44	50	52	63	64	56
Miyagi	42	51	58	62	68	60
Ōshima	42	47	51	51	58	53
Matsuyama	37	43	55	58	60	54
Kirishima	40	48	53	59	62	53
Miyazaki	42	50	54	60	59	55
Kagoshima	39	50	56	61	64	54
Average	41	48	54	59	62	55

According to YAMAGUCHI ('30) the majority of *P. agrestis* collected
in Sapporo possess the male openings in XVIII and I also met with
similar cases among collections made from Aomori prefecture. Though
individuals with male opening are rather rare in other localities so
far collected, their presence is certain. We noted similar cases in
most of the Japanese *Pheretima* which are normally destitute of the
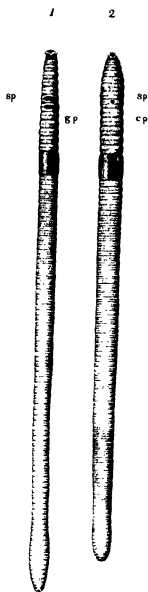
Fig 1
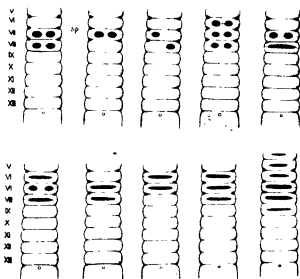
Fig 2
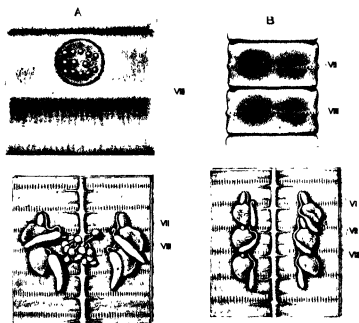
Fig 3

Fig 1   Showing the ventral surface of the entire body of *P agrestis* (1) and
P hilgendorfi (2) with a view to illustrating the forms of "patches" in these two
species   sp spermatheca openings, G P genital patches, C P Capsulogenous glands
   Fig 2   Showing the variations of the patches as to form, number and situation
of *P. agrestis*, collected from various parts of Japan   sp spermathecal openings
(The variations which occur in *P. hilgendorfi* as to the number and positions and
relative frequencies were carefully determined by YAMAGUCHI ('30))
   Fig. 3   Showing the enlarged pictures of the patches together with the form of
the capsulogenous gland and of the spermatheca in *P hilgendorfi* (A) and in *P agrestis*
(B).

male opening, cases with the male openings are often noted, as I ('29) have already mentioned in my report, on *Pheretima vittata*. *P. hilgendorfi* also shows a similar variation (OGAWA, '29). In subsequent reports, which will be published in the near future, I shall present similar cases in the majority of other species.

Pheretima yunoshimensis, nov. sp.

Thus far the present new species has been found only in the northern parts of Japan, especially in Hokkaido and Aomori perfectures. The species may easily be confused with *Pheretima hilgendorfi* as both possess a circular patch in segment VIII at identical positions and, furthermore, the spermathecal opening are found in VI/VII and VII/VIII. However when these two species are compared side by side, one will readily find that they are two distinct species, as will be seen from the differences in their respective body size and froms and in the form of the patches as well as in the arrangement and size of the papillae within the patches. An examination of the spermatheca as well as of the form of the capsulogenous glands at once indicates that these are two distinct species.

I shall now present the specific characters of the present new species.

Body length and breadth.

The body length varies considerably, but among the larger we find that it ranges from 130–145 mm., breadth about 6 mm., number of segments 87–98. Thus we see that the present species is rather slender and shorter than *P. hilgendorfi*. In small Yunoshima Island of Aomori prefecture the present species is abundantly found, together with *P. hilgendorfi*, *P. communissima*, *P. vittata* and several other *Pheretimas*, but one can easily tell it from *P. hilgendorfi* by its slender body and quicker movement without making any further structural examinations.

The first dorsal pore is found in XII/XIII. The spermathecal openings, as was already alluded, are two pairs in VI/VII, VII/VIII in the majority of cases, but I have seen some specimens with three pairs in V/VI, VI/VII, VII/VIII. I may add, however, that the pores in V/VI are usually unpaired, lacking in either side.

The number of setae are as follows:

Specimens collected from Yunoshima Island.

Segments.	No. 1.	No. 2.	No. 3.	No. 4.	
V	42	42	42	V	41
VI	49	46	50	VI	47
VII	51	51	56	VII	51
VIII	56	51	54	VIII	53
XX	60	60	60	IX	58
				X	61
				XI	61
				XVIII	58

The clitellum occupies the usual three segments XIV, XV and XVI and also lacks setae in these segments.

Male pores are usually lacking in the specimens in my hand but the specimens collected by YAMAGUCHI in Sapporo regularly possessed the male openings in XVIII. In fact, out of sixty four individuals collected at random in Yunoshima Island, Aomori prefecture, only one specimen possessed the male pores in XVIII.

The circular patches similar to those of *P. hilgendorfi* are found in the segments VIII and XVIII. The shape of the patch, when strictly speaking, is not so circular as in *P. hilgendorfi* but is somewhat oblong, as will be seen from Fig. 4, and the papillae within are much smaller and more numerous in the majority of cases. The patch of *P. hilgendorfi* is on the same level with the body surface, differing from that of *P. yunoshimensis* in which the patches are prominently elevated from the body surface and the margin of the patches is thicker and more prominently elevated. The patch in XVIII is invariably present and is more prominent than in VIII and is larger and, in addition, when fresh it presents a pink color while that of *P. hilgendorfi* (VIII) presents a yellowish brown. Since the body of this worm is slender while the patch in XVIII appears to occupy a greater part of the ventral area in XVIII. I may add that *P. hilgendorfi* possesses a patch in VIII only and seldom possesses any in XVIII. YAMAGUCHI ('30) recently examined a large number of *P. hilgendorfi* with a view to determining the extent of the variability in the number and positions of this "patch" or the capsurogenous glands in this earthworm and we can see from his observation that

cases with the patch in XVIII are 93 cases out of 1406, or only
about 7%. The form, and the regular occurrence in XVIII of the
patch, alone may distinguish this species from *P. hilgendorfi*
Internal characters.

The intestine begins at XV and in XXVII the six pairs of in-
testinal coeca are found. Nearer the dorsal, the size of the individual
coecum becomes longer and larger. The gizzards occupy VIII and
IX, and the septa VIII/IX and IX/X are missing. The sperm sacs are
in XI and XII, and the ovaries in XIII. The ovisacs are not found,
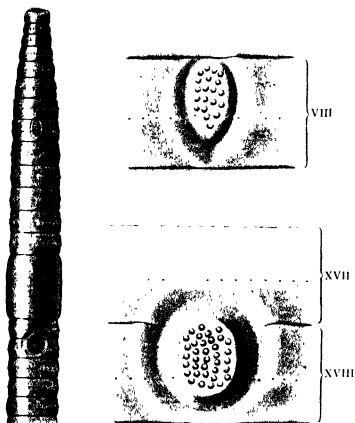
Fig 4   Ventral view of the anterior 19 segments showing the
forms and positions of the capsulogenous glands   Glands or patches in
segments VIII and XVIII are shown in enlarged figures

while these are comparatively well developed in *P. hilgendorfi.* The
prostate glands are wanting even when the male openings are present
while in the case of *P. hilgendorfi* they are invariably well developed
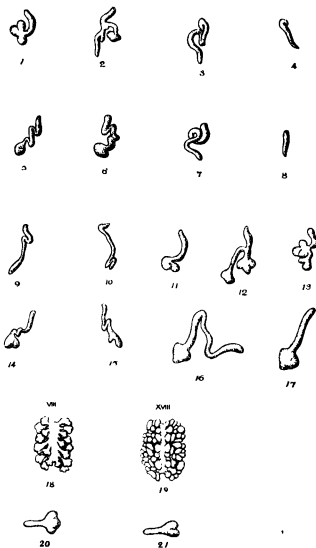
Fig 5. Showing various forms of spermatheca (1–17) together
with the capsulogenous glands in the segments VIII (18) and XVIII
(19), and component individual gland (20–21)

in the specimens with male openings. The spermatheca are unique and can most easily be distinguished from those of *P. hilgendorfi* (Fig. 5). The form of the main sac is irregularly con*toured and shows many protuberances, as the figure shows. In some instances a single tube of irregular shape is found, as the figures show, and in such cases one can not tell whether this tube is the diverticulum or the main saccular portion

The cap-ulogenous glands also differ from those found in *P. hilgendorfi* since the species here described possesses shorter stalked glands and, furthermore, the gland itself is not a regular spherical ball, as in the case of *P. hilgendorfi*, but is somewhat pointed at its tip with two or more deep inscisions, or is irregularly flat, as the figures show.

From the statements given above there is no question that this is a species distinct from *P. hilgendorfi*

Loc Yunoshima Island Aomori prefecture, and Sapporo, Hokkaido

Pheretima phaselus, nov sp

This is a very common earthworm living chiefly in gardens in Sendai and the northern parts of Japan. When fresh, it is nearly white all over the body with the exception of the chtellum which appears a deep purplish red When, however, preserved in formalin, the body surface becomes somewhat reddish, especially along the dorsal median line. The worm is slugish and does not make typical jump but instead secrets a mucous juice copiously.

The worm is stoutly built and the segments show secondary annulations. The chaeta are implanted over the elevated ridge as Fig. 6 shows.

The dorsal pores are distinct and begin in XII/XIII.

The spermathecal openings are found in V/VI, VI/VII, VII/VIII and there are no genital papilla in the neighborhood.

The ovidual pore is placed in a larger oblong-shaped are of a white color.

The male pores are most characteristic of this species, showing a large kidney bean shaped patch within which a longitudinal slit is found along its medium line. This slit is sometimes outwardly or

inwardly curved in the middle or, in some cases, nearly straight
Between the patches are found 14–16 setae.

The measurements of the body are as follows:

	Body length	Width	No	Segments
Sendai	103	4–5	32	102
Kominato	116	3–4	29	101
Sapporo	134	4–5	4	111
Asamushi	112	4–6	1	112
Shikoku	148	4–5	9	115
	123	4 5		108

The number of setate in the several segments are as follows
Kominato Series.

Segments	Specimen 1	Specimen 2	Average
V	42	45	44
VI	47	50	49
VII	53	55	54
VIII	38	56	57
XX	75	64	70

The septa are well developed especially in the genital region. The septum IX/X is lacking.

The spermatheca are found in VI, VII and VIII The saccular portion is pear shaped with a slender stalk similar to that shown in *P. maculosus*. The diverticulum is slender, with a slightly enlarged end It is usually curved or bent outwardly from the middle region.

There are no other glandular attachments or capsulogenous glands. which are very frequently found with most of the Japanese *Phere-timas*

The ovisac is present in XIII and is conspicuous.

Fig 6 Showing ventral view of anterior portion of body (A), sperma theca (B) and one prostate gland with duct (C)

The prostate gland is well developed consisting of three main lobes. The form of this spermiducal gland as a whole appears somewhat square and does not occupy many segments as it does in many other *Pheretimas*

The intestine begins in the segment XV and one pair of intestinal coeca is located in XXVII

Loc Sendai Aomori Prefecture Hokkaido Shikoku

Pheretima maculosus nov sp

In preserved specimens this species shows no noteworthy characteristics except perhaps a rather larger circular area around the male pores In formalin it appears slightly pinkish but the color of the clitellum is a deep purplish pink contrasted with the uniform light grayish body surface

When fresh this species is better characterised by its thinner, transparent body wall through which the intestinal and blood vessels are nearly well seen The dorsal surface is pigmented like other *Pheretima* with a lighter brick red color but the pigment is not distributed evenly all over the dorsal surface and extends irregularly and imperfectly with the exception of the narrow median dorsal line. When seen from the lateral the pigment sometimes extends lateral ward and then in the next segment it hardly covers even the dorsal surface alone Thus the marking of the body surface presents a spotted appearance and is very characteristic, distinguishing it from all other known *Pheretimas* in Japan (Fig 7)

The worm is rather medium sized and may be found under fallen leaves even during a cold frosty autumn and may even be found in early spring with well developed clitellum

This species was found in Aomori prefecture, Sapporo and Sendai

The spermathecal pores are three in V/VI VI/VII VII/VIII though not very distinct after preservation in formalin There are no genital papillae or any other structures of distinction

The ovidual pore is placed in a rather conspicuously depressed laterally oblong patch, the margin of which is lightly pigmented

The male openings are located within a circular well delineated patch within which from two to three small circular projections are noticed.

Between the male pore enumerated 12-13 setae.

The dorsal pores are indistinct in numerous cases and apparently begin in XIII/XIV. In many cases the dorsal pores are invisible in the clittelum, unlike most of the species belonging to *Pheretima* in which they are especially distinctly seen.

The measurements of the body of *P. maculosus* collected from various localities are as follows :

	Body length	Width	Segments.	Average of
Sendai	115	3-4	104	8
Sapporo	145	4-5	102	1
Kominato	125	3-4	102	7
Yokohama village.	94	4-5	83	1
Moura	98	3-4	97	5
Yunoshima	117	4-5	103	15
	105	3-4	106	29
	113	3-4	99	

The number of setae in several anterior segments of specimens collected in Yunoshima Island are as follows :

Segments.	Spec. 1	Spec 2
V	.3	45
VI	48	50
VII	53	55
''III	58	60
XX	67	69
Between male pores.	16	12

Internal characters.

The spermatheca are in three pairs and are located in VI, VII, and VIII. The general form of the spermatheca resembles that of *Pheretima phaselus* with a pear shaped saccular portion with a straight stalk. The diverticulum is slender, slightly shorter than the main pouch, and is curved loosely outwardly. (See Fig. 7, B).

In this species ovisacs are lacking, in contrast to the conspicuous ovisacs possessed by *Pheretima phaselus*. The ovaries are rather smaller when compared with the two species under consideration.

The prostate glands are well developed and the chief connection with the male duct occurs dorsally of the gland while in *Pheretima phaselus* such connection takes place on the ventral side of the spermiducal glands.

The intestine begins at XV and a single pair of the intestinal coeca originates in XXVII

The septa in the genital region, or XI/XII, and XII/XIII, are much thinner compared with the considerably thicker membrane on *Pheretima phaselus* in this same region

The septum is lacking in IX X and X XI

The present species closely resembles *Pheretima phaselus* but can easily be distinguished by the characteristic form of the patch surrounding the male openings which exhibits the shape of a kidney bean in *Pheretima phaselus* while in the present species it is a small circular papillae projection resembling that of *P communissima*, and furthermore the general body surface shows a diffuse distribution of pigment while the region where the pigment is lacking is transparent and the dorsal blood vessel and intestine can
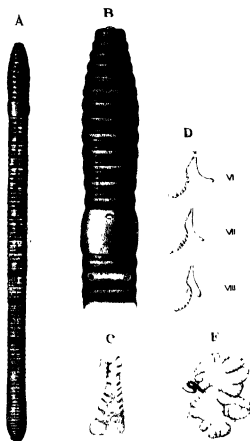
Fig 7 Showing the dorsal view of the entire body (A), ventral view anterior portion, more magnified (B), Intestinal Coecum (C), spermatheca (D) and Prostate gland with duct (E)

be seen through the body wall.

I may add that the *Pheretima maculosus* survives through winter and thus a worm with fully developed clitellum can be found at an early season before any other fully matured earthworms may be found.

Loc. Widely distributed in Aomori prefecture, Sapporo and Sendai.

Pheretima yamadai, nov sp

This very distinct species seems to be one commonly occuring in the central parts of Japan as I have seen this species years ago in collections made in Wakayama and Kobe and now I have received a very large number of these from both Tottori and Okayama through the courtesy of Prof. GENTARO YAMADA of the Tottori Goverment Agricultural College.

The species is stoutly built and is easily distinguishable owing to its possessing numerous segments for a comparatively shorter body, and also to its much enlarged prechtellar segments compared with the relatively narrow post-clitellar segments. The color is a typical brownish red dorsally and slightly lighter ventrally.

The spermathecal openings are found in VI/VII, VII/VIII, VIII/IX. In the orifice is found one or more conspicuous round papillae which are placed along the posterior edge of segments VI, VII and VIII To each papilla is attached internally a single pear shaped white gland. (See Fig. 8, C and E).

The ovidual pore is located in XIV

The male openings are characteristic of this species. In XVIII the male duct opens within a large round elevated papilla at the extreme lateral side and on the top of this papilla two or three minute papillae are found. In the interior, but near to the male duct, are found four round papillae, two upper and two immediately below and all these structures together form one large conspicuous area. In preserved specimens, the entire area, in which the male opening together with the four papilla are located, projects prominently from the body surface, as Fig. 8, A shows The setae are implanted within this large elevated area. The number of setae in between the male pores are very numerous, giving as many as 32, and in fact I have not yet encountered any other *Pheretima* in Japan which possesses so numerous

setae in this particular region To these four papillae are attached internally glandular structures.

The first dorsal pore begins in XII/XIII.

The body measurements are as follow.

	Length mm	Body		Segments
		Widest segments		
		Prechtellar	Postchtellar	
Average of 21 worm	127.1	6 5	4.6	98

The number of setae in serveral segments are as follows.

| | V | VI | VII | VIII | XX | Between male pores |
| Average of 13 worms | 63 | 68 | 71 | 71 | 72 | 32 |

The septa are well developed and, especially in the anterior segments, these are very thick The intestine begins in XV, and in XXVIII the finger shaped coeca with five projections are found in pairs

The shape of the spermatheca is well characterised having a very voluminous sac with a short stout stem. The diverticulum is attached close to the orifice of the stem and shows much winding though in some instances it shows much looser winding. The upper half of the diverticulum is somewhat more enlarged than the rest of the tube. In front of each spermatheca a pear-shaped or mushroom-shaped structure (probably of the same structure as the capsulogenous gland) is located. The number of these glandular bodies varies from one to two or probably more according to the individuals as well as in the different segments of the same individual, and the size of individual glands varies considerably. In all instances so far examined, these glands are located in front of the septum while the spermatheca itself is located to the rear of the septa concerned. The spermatheca are in VII, VIII and IX.

Sperm sacs are well developed. Although the ovaries are well developed while the ovisacs are apparently lacking.

The prostate glands are moderately large, occupying four segments. The glands are somewhat rectangular in shape with many lobes, as with most other species. The spermiducal duct is strongly developed and gradually enlarges its size as it nears the external orifice.

A

Slightly anterior and immediately near to the orifice, the four masses of the glandular structure corresponding to the four external papillae which are placed near the male pore, are located These structures just stated are not readily discernible as they are imbedded in and loosely covered by the muscle, or fibrous bundles, and furthermore their color in formalin preserved specimens is nearly the same as other surround tissues.

Loc   Tottori, Okayama, Wakayama, Kobe, and Hatta (Ishikawa prefecture).

B

D

E

C

g

pr g

Fig 8   Showing the ventral view of the anterior portion of the body (A), spermathecal openings (B) Enlarged male pore with papilla (C), Spermatheca with isolated gland and (D) Prostate gland with additional glands

g. The glands associated with the external papilla   pr. g – prostate gland

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Effect of Inorganic Salts on Photic Orientation in *Allolobophora foetida* (Sav.).

6. Magnesium Salts — MgSO₄, Mg(NO₃)₂, and MgCl₂.

BY

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(With 6 Text-figures)

ABSTRACT. The experiments were carried on by submerging the worms in a solution of the single or mixed magnesium salts.

1 In the ventral nerve cord, $MgSO_4$ and $Mg(NO_3)_2$ caused a strengthening at first and then a weakening of positively orienting functioning, while $MgCl_2$ caused from the beginning a slight weakening

2. In the brain, $MgSO_4$ caused a strengthening at first and then a weakening of negatively orienting functioning, while $Mg(NO_3)_2$ and $MgCl_2$ caused a weakening.

3 When $MgSO_4$ was mixed with $Mg(NO_3)_2$ or $MgCl_2$, the general tendency of change in orientation appeared mainly to follow that of the worms place I in $Mg(NO_3)_2$ or $MgCl_2$ respectively

4 When $Mg(NO_3)_2$ was mixed with $MgCl_2$, the change in positive orientation tended mainly to follow that occurring in $Mg(NO_3)_2$, but the change in negative orientation showed a tendency between those in $Mg(NO_3)_2$ and $MgCl_2$ separately.

5. In all the single and mixed solutions, an increased frequency of backward crawling after the submergence was caused by a relative weakening of forward crawling functioning in both the brain and the ventral nerve cord.

In our last preceding paper[5] we have described the effect of the sodium salts, Na_2SO_4, $NaNO_3$, and $NaCl$, on the photic orientation in *Allolobophora foetida*, and it was ascertained that among those salts only one, $NaCl$, could cause a strengthening of the degree of negative orientation in the worms, even after a prolonged submergence

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[1] $MgCl_2$, $CaCl_2$, $NaCl$, and KCl. Sci. Rep. Tôhoku Imp. Univ., 4th Ser., Vol. 3, No. 2, Pp. 151–177. Methods of the experiments and of treating data are given in this paper.

[2] $MgSO_4$, $FeSO_4$, Na_2SO_4 and K_2SO_4. Ibid., Vol. 3, No. 3, Fasc. 1, Pp. 223–248.

[3] $Mg(NO_3)_2$, $Ca(NO_3)_2$, $NaNO_3$, and KNO_3. Ibid., Vol. 3, No. 3, Fasc. 2, Pp. 379–403.

[4] NaI, KI, $NaBr$, and KBr. Ibid., Vol. 3, No. 4, Fasc. 1, Pp. 647–663.

[5] Sodium Salts, Na_2SO_4, $NaNO_3$, and $NaCl$. Ibid., Vol. 5, No. 3, Pp. 467–483.

in its solution. Among other salts, however, the effects of which were already studied[1-4], we found one, $MgCl_2$, which, contrary to the case of NaCl, tended to cause a strengthening of positively orienting functioning in the ventral nerve cord and a weakening of negatively orienting functioning in the brain, therefore a weakening of the degree of negative orientation in the worms as a whole with the lapse of time. The experiments, which are to be noted in the present paper, were therefore undertaken to test again the effect of $MgCl_2$ and, in connection with this, of the other magnesium salts, $MgSO_4$ and $Mg(NO_3)_2$.

The control experiments were carried on in a temperature of 23 C. on the 14th day of July, 1928, and again in a temperature of 25°C. on the 26th of the same month. The data used in this paper were the averages obtained from these two sets of experiments.

I   EFFECT OF SINGLE SALTS

The experiments were carried on in a temperature of 23–25 C., July 14–20, 1928. In the experiments, 1/2 the normal solutions of $MgSO_4$, $Mg(NO_3)_2$, and $MgCl_2$ were used.

A. MOVEMENTS OF UNOPERATED WORMS.

50 worms were tested individually in a definite solution for each duration of submergence, viz. 30, 60, 90 or 120 seconds.

In $MgSO_4$, the worms showed neither convulsion nor ejection of coelomic fluid in coincidence with the result of previous experiments, and even at a duration of submergence above 500 seconds they were still active.

In $Mg(NO_3)_2$, the worms showed convulsion at the beginning of submergence. Ejection of coelomic fluid, however, was observed only after a lapse of above 50 seconds. The worms became so sluggish at a duration of above 110 seconds, that we could not obtain the data at 120 seconds.

In $MgCl_2$, the worms showed convulsion at 80–100 seconds, but ejection of coelomic fluid was not observed even at 230 seconds, at which time most of the worms became almost inactive.

1—4) Loc. cit

The differences in number of seconds found between the present and previous experiments may depend upon the difference of the month,

TABLE 1.

Angles occupied by the unoperated worms.

Duration of submergence in seconds	5 cm angles in degrees			10 cm angles in degrees		
	Positive	Average	Negative	Positive	Average	Negative
MgSO₄						
0	85.24	111.62	116.38	84.16	122.64	128 48
30	79.40	113.16	128 76	79.46	125.24	125.78
60	77.80	106.78	118.98	76 58	113.90	127.32
90	78.78	104 74	115.96	77.04	108.92	121.88
120	76.40	92 86	106.46	72.48	91.06	108.58
Mg(NO₃)₂						
0	85.24	111.62	116.38	84.16	122 64	128.48
30	80.20	101 16	110.96	80.94	118 56	127.42
60	80.80	101.24	110.44	80 70	112 04	121.34
90	68.40	91 16	112.76	66 54	93.46	116.92
MgCl₂						
0	85 24	111 62	116.38	84.16	122.64	128.48
30	79.96	98.52	108 56	78.16	105 26	117.10
60	79.56	95.22	103.66	77 88	94 06	106.18
90	80.54	91.00	100.46	77 02	92.66	105.64
120	75.62	82.14	96.52	77.04	89.76	102.72

TABLE 2.

Frequency distribution of the unoperated worms

Duration of submergence in seconds	5 cm angles			10 cm angles		
	0°-80°	81°-99°	100°-180°	0°-80°	81°-99°	100°-180°
MgSO₄						
0	6	13	31	9	6	35
30	12	7	31	11	3	36
60	17	5	28	15	5	30
90	18	4	28	17	6	27
120	18	13	19	20	9	21
Mg(NO₃)₂						
0	6	13	31	9	6	35
30	16	8	26	11	5	34
60	16	9	25	12	5	33
90	26	2	22	22	1	27
MgCl₂						
0	6	13	31	9	6	35
30	13	12	25	14	8	28
60	16	13	13	19	10	21
90	18	15	17	18	10	22
120	24	13	13	20	10	20

of the degrees of temperature, and of the concentration.

Orientation.

According to Tables 1 and 2, in $MgSO_4$ the degree of negative orientation of the worms tended to strengthen at first and then to weaken with the increase of the number of seconds of submergence, while in $Mg(NO_3)_2$ it tended to weaken The change in $MgCl_2$ shows a similar tendency with the result of the previous experiments, but those in $MgSO_4$ and $Mg(NO_3)_2$ show utterly different tendencies.

Crawling.

It may be stated from Table 3 that in all the solutions, especially in $Mg(NO_3)_2$, the numbers of backward crawling and winding indivi-

TABLE 3.

Frequency of crawling of the unoperated worms.

| | Duration of sub- mergence in seconds | 5 cm angles | | | | | 10 cm. angles | | | |
| | | Forward | | | Backward | | | | | |
		Directly	After posterior elongation		Directly	After anterior elongation		Forward	Backward	Returning	Winding
MgSO₄	0	50	0		0	0		50	0	0	0
	30	43	0		7	0		44	6	0	0
	60	48	0		2	0		48	2	0	1
	90	50	0		0	0		50	0	0	0
	120	47	0		3	0		47	3	0	1
Mg(NO₃)₂	0	50	0		0	0		50	0	0	0
	30	44	2		4	0		47	3	3	1
	60	42	2		6	0		44	6	1	1
	90	32	2		16	0		35	15	0	5
MgCl₂	0	50	0		0	0		50	0	0	0
	30	50	0		0	0		50	0	0	0
	60	46	0		4	0		46	4	0	2
	90	50	0		0	0		50	0	1	1
	120	49	0		1	0		49	1	0	0

duals were increased after the submergence. The number of returning individuals was also increased in Mg(NO$_3$)$_2$ and MgCl$_2$. These tendencies are in general coincidence with the results of the previous experiments.

B. MOVEMENTS OF OPERATED WORMS.

25 worms were tested individually for each duration of submergence.

Orientation.

From Tables 4 and 5 the following tendencies may be noted:

In MgSO$_4$ and Mg(NO$_3$)$_2$, the degree of positive orientation of the worms tended to strengthen at first and then to weaken with the prolongation of the duration of submergence. This tendency appears alike to that which was noted in the preceding paper.

In MgCl$_2$, the degree of positive orientation tended rather to weaken. This tendency is never alike to that which was noted formerly.

TABLE 4.

Angles occupied by the operated worms.

	Duration of submergence in seconds	5 cm. angles in degrees			10 cm angles in degrees		
		Positive	Average	Negative	Positive	Average	Negative
MgSO$_4$	0	49.10	53.00	93.90	39.28	45.02	95.74
	30	49.60	53.64	94.04	35.12	42.24	97.12
	60	57.52	62.76	95.24	45.20	56.48	101.28
	90	61.52	68.72	97.20	49.20	58.78	99.56
	120	67.64	73.76	96.12	66.64	76.76	100.12
Mg(NO$_3$)$_2$	0	49.10	55.00	92.90	39.28	45.02	95.74
	30	48.24	52.84	94.60	38.24	42.84	94.60
	60	53.12	55.04	92.52	39.80	46.00	96.20
	90	55.36	83.48	98.12	50.36	59.60	99.24
MgCl$_2$	0	49.10	53.00	93.90	39.28	45.02	95.74
	30	50.92	55.40	95.48	44.48	49.84	95.36
	60	55.20	·57.88	92.68	42.72	46.40	93.68
	90	58.52	63.16	94.84	46.52	53.92	96.40
	120	57.12	61.44	94.32	43.28	48.20	94.92

TABLE 5.

Frequency distribution of the operated worms.

	Duration of submergence in seconds	5 cm angles			10 cm angles		
		0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
MgSO₄	0	19	3	3	20	1	4
	30	20	2	3	22	0	3
	60	15	5	5	19	2	4
	90	14	6	5	18	2	5
	120	14	3	8	13	4	8
Mg(NO₃)₂	0	19	3	3	20	1	4
	30	21	3	1	22	0	3
	60	18	2	5	19	3	3
	90	16	4	5	18	2	5
MgCl₂	0	19	3	3	20	1	4
	30	16	4	5	19	2	4
	60	20	3	2	20	2	3
	90	18	4	3	19	2	4
	120	17	5	3	20	2	3

Crawling.

In all the cases (Table 6) the number of backward crawling individuals was increased after the submergence. The number of winding individuals might also be increased.

TABLE 6

Frequency of crawling of the operated worms.

	Duration of submergence in seconds	5 cm angles					10 cm angles			
		Forward			Backward					
		Directly	After posterior elongation		Directly	After anterior elongation		Forward	Backward	Returning	Winding
MgSO₄	0	14	2		9	0		17	8	0	0
	30	0	0		25	0		0	25	0	0
	60	4	0		21	0		4	21	0	0
	90	1	1		22	1		2	23	0	0
	120	0	2		14	0		11	14	0	0

| | Duration of submergence in seconds | 5 cm angles | | | | 10 cm angles | | | |
| | | Forward | | Backward | | | | | |
		Directly	After posterior elongation	Directly	After anterior elongation	Forward	Backward	Returning	Winding
Mg(NO₃)	0	14	2	9	0	17	8	0	0
	30	9	3	13	0	13	12	0	0
	60	15	1	9	0	17	8	0	1
	90	18	2	5	0	21	4	0	0
MgCl₂	0	14	2	9	0	17	8	0	0
	30	6	2	16	1	8	17	0	0
	60	2	1	21	1	3	22	0	1
	90	7	1	17	0	8	17	0	0
	120	13	0	11	1	13	12	0	1

C. Changes of Negativity in the Brain.

From Table 7, Figs. 1-3 were plotted in order to see easily the states of the changes. In these figures the tracings of the 5 cm. angles are denoted by the broken lines, and those of the 10 cm angles by the full lines.

TABLE 7.

Calculation of N.

| | Duration of submergence in seconds | 5 cm. angles in degrees | | | 10 cm angles in degrees | | |
		P	A	N	P	A	N
MgSO₄	0	53.00	111.62	148.62	45.02	122.64	167.62
	30	53.64	113.16	149.52	42.24	125.24	173.00
	60	62.76	106.78	134.02	56.48	113.90	147.42
	90	68.72	104.74	126.92	58.76	108.92	140.16
	120	73.76	92.86	109.10	76.76	91.06	104.30
Mg(NO₃)₂	0	53.00	111.02	148.62	45.02	122.64	167.62
	80	52.84	101.16	138.32	42.84	118.36	165.52
	60	55.64	101.24	135.60	46.00	112.04	156.04
	90	63.48	91.16	117.68	59.60	93.46	123.86
MgCl₂	0	53.00	111.62	148.62	45.02	122.64	167.62
	80	65.40	98.52	123.12	49.84	105.26	145.42
	60	57.88	93.22	125.34	46.40	94.06	137.66
	90	63.16	91.00	117.84	52.92	92.66	129.74
	120	61.44	82.14	110.70	48.20	89.76	131.56

MgSO₄ (Fig. 1) tended to cause in the brain a strengthening at first and then a weakening of the degree of negative orientation. This

Fig. 1   MgSO₄.
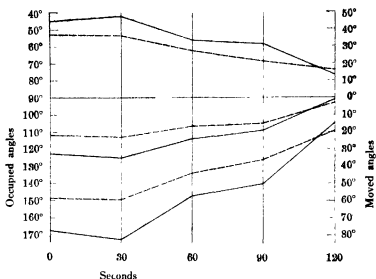
Fig. 2   Mg(NO₃)₂
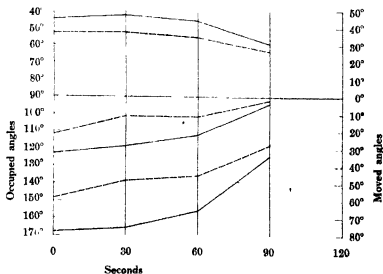
Fig. 3. MgCl₂

Fig. 3. $MgCl_2$
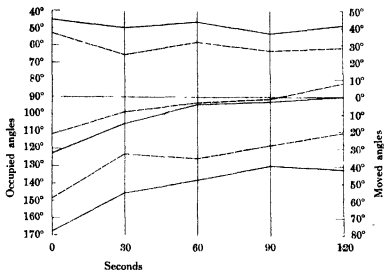
tendency utterly differs from that shown in the previous experiments.

$Mg(NO_3)_2$ (Fig. 2) and $MgCl_2$ (Fig. 3) tended to cause in the brain a weakening of the degree of negative orientation. This tendency coincides with the respective result obtained in the preceding experiments.

D. CHANGES IN CRAWLING.

From Tables 3 and 6 we may infer that, in all the solutions, the strengthening of backward crawling was caused by a relative weakening of forward crawling functioning in both the brain and the ventral nerve cord. The changes in $Mg(NO_3)_2$ and $MgCl_2$ coincide with those noted in the previous papers. As to the disagreement of the changes in $MgSO_4$, we can at present only state that a relative weakening of forward crawling functioning in the brain may be also a factor causing a strengthening of backward crawling of the worms.

II EFFECT OF MIXED SALTS.

The experiments were carried on in a temperature of 24°-25°C., July 20-27, 1928. In the experiment, an amount of one of 1/2 normal

solutions of $MgSO_4$, $Mg(NO_3)_2$, and $MgCl_2$ was mixed with the same amount of one of the other solutions.

A. Movements of Unoperated Worms.

50 worms were tested individually for each duration of submergence.

In all the mixed solutions, the worms showed neither convulsion nor ejection of coelomic fluid.

In $MgSO_4 + Mg(NO_3)_2$ at a duration of submergence of above 250 seconds, and in $Mg(NO_3)_2 + MgCl_2$ at above 130 seconds, the worms became very sluggish, while in $MgSO_4 + MgCl_2$ they were still active even after a lapse of 500 seconds.

Orientation.

From Tables 8 and 9 we may infer that in all the mixed solutions, the worms tended to show a weakening of the degree of negative orientation. In $MgSO_4 + Mg(NO_3)_2$ this tendency of change may be understood as an expression between those shown by the worms placed

TABLE 8.

Angles occupied by the unoperated worms.

Duration of submergence in seconds	5 cm. angles in degrees			10 cm angles in degree		
	Positive	Average	Negative	Positive	Average	Negative
$MgSO_4$ + $Mg(NO_3)_2$						
0	85.24	111.62	116.38	84.16	122.64	128.48
30	79.52	98.60	109.08	77.08	109.62	121.64
60	76.68	92.96	106.28	75.84	101.46	115.62
90	71.36	86.36	105.00	70.86	92.50	111.64
120	75.62	87.74	102.12	68.94	88.94	110.00
$MgSO_4$ + $MgCl_2$						
0	85.24	111.62	116.38	84.16	122.64	128.48
30	77.66	98.28	110.62	75.80	107.60	121.80
60	76.74	96.12	109.38	77.10	104.70	117.60
90	77.52	92.86	105.34	79.94	102.02	112.08
120	80.62	91.28	100.66	79.66	97.26	107.60
$Mg(NO_3)_2$ + $MgCl_3$						
0	85.24	111.62	116.38	84.16	122.64	128.48
30	77.94	92.90	104.96	76.42	97.46	111.04
60	75.18	88.20	103.02	74.44	92.24	107.80
90	69.34	83.02	103.68	75.76	90.16	104.40
120	73.58	87.36	103.78	75.54	91.88	106.34

TABLE 9.

Frequency distribution of the unoperated worms.

Duration of submergence in seconds	5 cm angles			10 cm angles		
	0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
MgSO₄ + Mg(NO₃)₂						
0	6	13	31	9	6	35
30	17	9	24	15	9	26
60	21	8	21	16	9	25
90	26	7	17	23	5	22
120	24	10	16	25	5	20
MgSO₄ + MgCl₂						
0	6	13	31	9	6	35
30	19	5	26	18	2	30
60	18	8	24	16	7	27
90	17	13	20	15	10	25
120	17	20	13	14	15	21
Mg(NO₃)₂ + MgCl₂						
0	6	13	31	9	6	35
30	15	12	23	17	7	26
60	22	11	17	24	4	22
90	25	9	16	25	5	20
120	24	8	18	23	4	23

separately in MgSO₄ and Mg(NO₃)₂, and in MgSO₄+MgCl₂ and Mg(NO₃)₂+MgCl₂ this tendency appears rather to resemble that in MgCl₂.

Crawling.

In all the cases (Table 10) the numbers of backward crawling and winding individuals were increased after the submergence.

B. MOVEMENTS OF OPERATED WORMS.

25 worms were used individually.

Orientation.

From Tables 11 and 12 the following tendencies may be noted:

In MgSO₄+Mg(NO₃)₂ the worms showed a strengthening at first and then a weakening of the degree of positive orientation. This tendency of change may be taken as an expression between those of the worms placed in MgSO₄ and Mg(NO₃)₂ separately.

TABLE 10.

Frequency of crawling of the unoperated worms.

Duration of submergence in seconds	5 cm angles				10 cm angles		Returning	Winding
	Forward		Backward		Forward	Backward		
	Directly	After posterior elongation	Directly	After anterior elongation				
0	50	0	0	0	50	0	0	0
30	50	0	0	0	50	0	0	1
60	47	0	3	0	46	4	0	0
90	50	0	0	0	49	1	0	0
120	46	0	4	0	46	4	0	0
0	50	0	0	0	50	0	0	0
30	50	0	0	0	50	0	0	0
60	49	0	1	0	50	0	1	0
90	49	1	0	0	50	0	0	1
120	48	0	2	0	48	2	0	0
0	50	0	0	0	50	0	0	0
30	49	0	0	1	49	1	0	0
60	50	0	0	0	50	0	0	0
90	43	0	7	0	45	5	0	1
120	49	0	1	0	49	1	0	0

(Row groups: $MgSO_4$ + $Mg(NO_3)_2$; $MgSO_4$ + $MgCl_2$; $Mg(NO_3)_2$ + $MgCl_2$)

TABLE 11.

Angles occupied by the operated worms.

	Duration of submergence in seconds	5 cm angles in degrees			10 cm angles in degrees		
		Positive	Average	Negative	Positive	Average	Negative
$MgSO_4$ + $Mg(NO)_2$	0	49.10	53.00	93.90	39.28	45.02	95.74
	30	46.12	49.66	93.54	37.48	41.24	93.76
	60	49.04	51.92	92.88	41.84	44.68	92.84
	90	60.52	63.23	92.71	47.76	50.64	92.88
	120	65.08	68.76	93.68	46.68	50.80	94.12
$MgSO_4$ + $MgCl_2$	0	49.10	53.00	93.90	39.28	45.02	95.74
	30	51.72	56.24	94.52	42.44	45.84	93.40
	60	56.76	59.28	92.52	41.24	45.12	93.88
	90	60.52	64.96	94.44	45.24	48.36	93.12
	120	59.20	68.24	99.04	48.68	54.28	95.60
$Mg(NO_3)_2$ + $MgRCl_2$	0	49.10	53.00	93.90	39.28	45.02	95.74
	30	46.40	48.92	92.52	33.80	37.56	93.76
	60	55.00	57.04	92.04	46.32	50.04	93.72
	90	57.16	62.16	95.00	48.48	55.16	96.48
	120	58.84	64.04	95.20	59.44	66.44	97.00

TABLE 12.

Frequency distribution of the operated worms.

	Duration of submergence in seconds	5 cm angles			10 cm angles		
		0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
MgSO₄ + Mg(NO₃)₂	0	19	3	3	20	1	4
	30	21	2	2	21	2	2
	60	19	3	3	20	2	3
	90	16	7	2	18	4	3
	120	14	7	4	16	5	4
MgSO₄ + MgCl₂	0	19	3	3	20	1	4
	30	20	3	2	22	1	2
	60	20	3	2	20	3	2
	90	19	2	4	20	2	3
	120	16	4	5	18	4	3
Mg(NO₃)₂ + MgCl₂	0	19	3	3	20	1	4
	30	20	2	3	23	0	2
	60	18	5	2	20	3	2
	90	18	4	3	19	2	4
	120	18	3	4	16	4	5

TABLE 13.

Frequency of crawling of the operated worms.

	Duration of submergence in seconds	5 cm angles				10 cm angles			
		Forward		Backward					
		Directly	After posterior elongation	Directly	After anterior elongation	Forward	Backward	Returning	Winding
MgSO₄ + Mg(NO₃)₂	0	14	2	9	0	17	8	0	0
	30	3	2	20	0	5	20	0	0
	60	4	2	18	1	6	19	0	0
	90	6	1	17	1	8	17	0	1
	120	6	1	17	1	8	17	0	1
MgSO₄ + MgCl₂	0	14	2	9	0	17	8	0	0
	30	4	0	18	3	4	21	0	0
	60	9	0	15	1	9	16	0	1
	90	2	0	23	0	3	22	0	1
	120	4	3	18	0	7	18	0	2
Mg(NO₃)₂ + MgCl₂	0	14	2	9	0	17	8	0	0
	30	9	0	15	1	8	17	0	0
	60	8	1	16	0	9	16	0	0
	90	13	3	9	0	17	8	0	0
	120	15	0	8	2	15	10	0	1

In $Mg(NO_3)_2 + MgCl_2$, the worms showed also a tendency similar to the above, and this tendency appears to resemble that in $Mg(NO_3)_2$.

In $MgSO_4 + MgCl_2$, the worms showed a weakening of the degree of positive orientation. This tendency appears rather to resemble that in $MgCl_2$.

Crawling

From Table 13 we may infer that in the mixed solution the numbers of backward crawling and winding individuals were increased after the submergence.

C. CHANGES OF NEGATIVITY IN THE BRAIN.

From Table 14, Figs. 4–6 were plotted

In all the cases, the degree of negative orientation in the brain showed a weakening, following the prolongation of the duration of submergence.

In $MgSO_4 + Mg(NO_3)_2$ (Fig. 4) or $MgSO_4 + MgCl_2$ (Fig. 5), the

TABLE 11.

Calculation of N.

	Duration of submergence in seconds	5 cm angles in degrees			10 cm angles in degrees		
		P	A	N	P	A	N
$MgSO_4$ + $Mg(NO_3)_2$	0	53.00	111.62	148.62	45.02	122.64	167.62
	30	49.66	98.60	138.94	41.24	109.62	158.88
	60	51.92	92.96	131.04	44.68	101.46	146.78
	90	63.23	86.36	113.13	50.64	92.50	131.86
	120	68.76	87.7¾	108.98	60.80	88.94	118.14
$MgSO_4$ + $MgCl_2$	0	53.00	111.62	148.62	45.02	122.64	167.62
	30	56.24	98.28	132.04	45.84	107.60	151.76
	60	59.28	96.12	126.84	45.12	104.70	149.58
	90	64.96	92.86	117.90	48.36	102.02	143.66
	120	68.24	91.28	113.04	54.28	97.26	132.98
$Mg(NO_3)_2$ + $MgCl_2$	0	53.00	111.62	148.62	45.02	122.64	167.62
	30	48.92	92.90	133.98	37.56	97.46	149.90
	60	57.04	88.20	121.16	50.04	92.24	132.20
	90	62.16	83.02	110.86	55.16	90.16	125.00
	120	64.04	87.36	113.32	66.44	91.88	115.44

tendency of change appears to be influenced by $Mg(NO_3)_2$ or $MgCl_2$ respectively more than by $MgSO_4$, and in $Mg(NO_3)_2$ · $MgCl_2$ (Fig. 6), it may be taken as an expression between those of the worms placed in $Mg(NO_3)_2$ and $MgCl_2$ separately.

Fig. 4 $MgSO_4+Mg(NO_3)_2$.
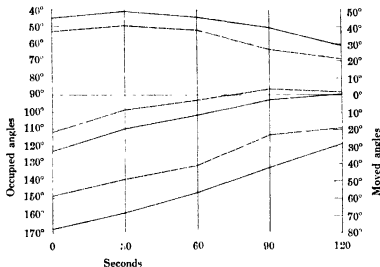
Fig. 5 $MgSO_4+MgCl_2$
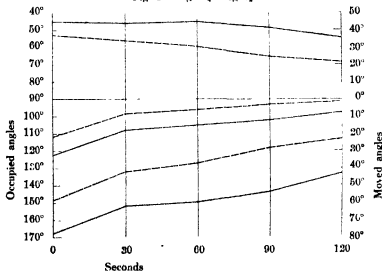
Fig. 6. $Mg(NO_3)_2 + MgCl_2$.
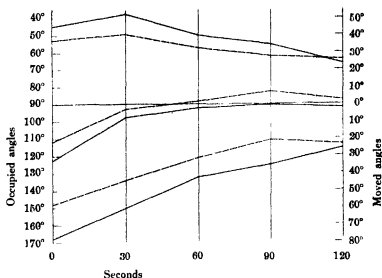
D. CHANGES IN CRAWLING.

From Tables 10 and 13 it may be inferred that in the mixed solutions used, the strengthening of backward crawling was caused by a relative weakening of forward crawling functioning in both the brain and the ventral nerve cord.

III. REMARKS

$Mg(NO_3)_2$.   Let us first make mention of $Mg(NO_3)_2$. The previous set of experiments was carried on in a temperature of 15.6°–16.0°C., October 16–21, while the present set was under an influence of temperature 23°–25°C., July 14–20, though in both sets a solution of the same concentration, namely 1/2 normal solution, was used. Probably because of the difference of the degrees of temperature as well as of the seasons, we obtained as the result of the new experiments a tendency of change in A or in the degree of photic orientation of the unoperated worms, which is different from that of the previous. According to our hypothesis, this difference is due to the difference in the antagonistic relation occurring in the respective set between P,

or the degree of positive orientation shown by the ventral nerve cord,
and *N*, or the degree of negative orientation shown by the brain.
In fact, in the new experiments the tendency of change of *P* and of
N was more or less modified from the respective tendency obtained
in the previous, even though in both sets these were fundamentally
identical. This instance therefore appears to us to be a suitable case
in suggesting that the tendencies of change in the brain and the
ventral nerve cord must be determined as we are doing before the
validity of reversals of photic orientation of the worms is spoken of
under various conditions.

MgCl₂. In the previous experiments, 1/2 the normal solution was
used in a temperature of about 20°C., June 10–22, and the operated
worms (*P*) showed a tendency to strengthening the degree of positive
orientation   The present experiments however were carried on under
an influence of a little higher temperature, July 14–20, and the
operated worms showed a tendency to rather weakening the degree
of positive orientation, in spite of placing them also in a solution of
the same concentration. As to the difference between the tendencies
of change in *Ps*, we are of the opinion at present that it is mainly
due to the different degrees of temperature as well as to the different
months[6], and we proceed to state the facts as they were without
adding further supposition.

MgSO₄. In the previous set of experiments, 1/1.7 the normal
solution was used under an influence of temperature of 22.8°–25.6°C.,
September 2–23, and in the present set, 1/2 the normal solution was
used in a temperature of 23°–25°C., July 14–20. Thus it may be
considered that both experiments were conducted under nearly the
same conditions, in spite of the difference of the concentrations as
well as of the seasons[6]. Nevertheless, the result from *A* and from *N*
in the respective set was so different from each other, that it was
impossible to us to treat this case so briefly as in the cases of
Mg(NO₃)₂ and MgCl₂. In order to test again, therefore, the effect of
MgSO₄, another set of experiments was made in a temperature of
17°C., May 23–24, 1930, using also 1/2 the normal solution. The

6) E. NOMURA and S. OHFUCHI. ,Seasonal changes of photic orientation in *Allolo-*
    bophora foetida. 1928. Sci. Rep. Tôhoku Imp. Univ., 4th Ser., Vol 3, No. 2, Pp
    97–112.

TABLE 15.

Records from 50 unoperated worms placed in MgSO₄

	Duration of submergence in seconds	5 cm angles in degrees			10 cm. angles in degree		
		Positive	Average	Negative	Positive	Average	Negative
Angles occupied	0	82.40	104.10	111.70	84.04	122.20	128.16
	30	82.96	104.96	112.00	82.48	123.46	130.98
	60	82.57	106.30	113.73	83.38	126.22	132.84
	90	80.08	102.48	113.40	83.14	119.10	125.96
	120	76.86	90.96	104.10	82.66	108.22	115.66
	180	75.84	90.66	104.82	78.16	104.16	116.00
	240	74.34	90.12	105.78	81.18	105.76	114.58

	Duration of submergence in seconds	5 cm angles			10 cm angles		
		0°-80°	81°-99°	100°-180°	0°-80°	81°-99°	100°-180°
Frequency distribution	0	11	14	25	8	6	36
	30	10	15	25	10	3	37
	60	11	13	26	8	4	38
	90	15	9	26	9	7	34
	120	20	7	23	17	5	28
	180	20	9	21	17	6	27
	240	19	11	20	16	6	28

	Duration of submergence in seconds	5 cm angles				10 cm angles			
		Forward		Backward					
		Directly	After posterior elongation	Directly	After anterior elongation	Forward	Backward	Returning	Winding
Frequency of crawling	0	50	0	0	0	50	0	8	0
	30	46	0	4	0	46	4	3	0
	60	48	0	2	0	48	2	4	0
	90	49	0	1	0	49	1	1	0
	120	48	0	2	0	48	2	0	0
	180	50	0	0	0	50	0	0	0
	240	49	0	1	0	49	1	0	0

results are recorded in Tables 15–17, and from these tables the
following tendencies may be inferred:

1. In the unoperated worms (Table 15), the degree of negative
orientation tended to strengthen at first and then to weaken with the
prolongation of the duration of submergence, and in the operated
worms (Table 16), the degree of positive orientation tended also to

TABLE 16.

Records from 25 operated worms placed in MgSO₄.

	Duration of submergence in seconds	5 cm. angles in degrees			10 cm. angles in degrees		
		Positive	Average	Negative	Positive	Average	Negative
Angles occupied	0	62.28	70.60	98.32	55.76	69.44	103.68
	30	50.64	55.56	94.92	38.08	46.52	98.44
	60	54.44	58.56	94.12	35.28	41.52	96.04
	90	56.84	61.72	94.88	38.96	43.28	94.32
	120	58.68	62.04	93.36	45.24	50.12	94.88
	180	67.84	70.80	92.96	57.32	62.36	95.04
	240	65.36	68.20	92.84	58.28	67.48	99.20

	Duration of submergence in seconds	5 cm angles			10 cm angles		
		0°–80°	81°–99°	100°–180°	0°–80°	81°–99°	100°–180°
Frequency distribution	0	17	3	5	18	1	6
	30	20	2	3	21	0	4
	60	18	3	4	22	1	2
	90	17	5	3	21	2	2
	120	16	5	4	19	2	4
	180	13	7	5	15	4	6
	240	14	6	5	15	3	7

	Duration of submergence in seconds	5 cm angles				10 cm angles			
		Forward		Backward					
		Directly	After posterior elongation	Directly	After anterior elongation	Forward	Backward	Returning	Winding
Frequency of crawling	0	19	1	3	2	20	5	0	0
	30	14	4	7	0	18	7	0	0
	60	16	0	7	2	16	9	0	1
	90	12	3	9	1	10	15	0	0
	120	17	0	6	2	17	8	0	1
	180	16	1	7	1	18	7	0	1
	240	9	1	15	0	11	14	0	0

strengthen at first and then to weaken. Thus we learn that in the brain (Table 17) the degree of negative orientation tended to strengthen at first and then to weaken.

2. In both the operated and unoperated worms, the number of backward crawling individuals was increased after the submergence. Thus it may be stated that the increased frequency of backward

TABLE 17.

Calculation of N from the records made in MgSO₄.

Duration of submergence in seconds	5 cm. angles in degrees			10 cm. angles in degrees		
	P	A	N	P	A	N
0	70 60	104.10	123.50	69.44	122.20	142.76
30	55.56	104.96	139.40	46.52	123.46	166.94
60	58.56	106.30	137.74	41.32	126.22	174.90
90	61.72	103.48	131.76	43.28	119.10	165.82
120	62.04	90.96	118.92	50.12	108 32	148 20
180	70 80	90.66	109.86	62.86	104.16	131.80
240	68.20	90.12	111.92	67.48	105.76	128.28

crawling is caused by a relative weakening of forward crawling functioning in both the brain and the ventral nerve cord.

From the results just stated, we come now to believe that the previous series of experiments may contain some errors; and from the evidences occurring in the mixed solutions containing MgSO₄ not only in the present but also in the previous experiments, we come to accept the correctness of the new results. The corrigenda relating to this point will be given later at the end of this report.

IV SUMMARY.

MgSO₄ caused a strengthening at first and then a weakening of both positively orienting functioning in the ventral nerve cord and negatively orienting functioning in the brain.

Mg(NO₃)₂, MgSO₄ + Mg(NO₃)₂, and Mg(NO₃)₂ + MgCl₂ caused a strengthening at first and then a weakening of positively, and a weakening of negatively, orienting functioning.

MgCl₂, and MgSO₄ + MgCl₂ caused a weakening of both positively and negatively orienting functionings.

When MgSO₄ was mixed with Mg(NO₃)₂ or MgCl₂, the general tendency of change in orientation appeared mainly to follow that of the worms placed in Mg(NO₃)₂ or MgCl₂ respectively.

In Mg(NO₃)₂ + MgCl₂, the change in positive orientation tended mainly to follow that occurring in Mg(NO₃)₂, but the change in negative orientation showed a tendency between those in Mg(NO₃)₂ and MgCl₂ separately.

In Mg(NO₃)₂, convulsion occurred at the beginning of submergence and ejection of coelomic fluid at above 50 seconds.

In MgCl₂, convulsion occurred at 80–100 seconds of submergence, but ejection of coelomic fluid did not occur.

In MgSO₄, MgSO₄+Mg(NO₃)₂, MgSO₄+MgCl₂, and ?Mg(NO₃)₂+MgCl₂ neither convulsion nor ejection of coelomic fluid occurred.

In all the single and mixed solutions, the increased frequency of backward crawling after the submergence was caused by a relative weakening of forward crawling functioning in both the brain and the ventral nerve cord.

In MgSO₄, returning movement was weakened after the submergence, while winding movement was strengthened.

In Mg(NO₃)₂ and MgCl₂, both returning and winding movements were more or less strengthened.

Corrigenda.

The corrigenda are given to correct the misinterpretations stated in the previous report[2] in connection with the effects of the mixed salts containing MgSO₄.

1. In MgSO₄+FeSO₄, the general tendency of change in orientation tended mainly to follow that of the worms placed in MgSO₄.

2. In MgSO₄+Na₂SO₄, the general tendency of change in orientation appeared to be an expression between those in MgSO₄ and Na₂SO₄ separately.

3. In MgSO₄+K₂SO₄, in the 5 cm. angles the general tendency of change tended mainly to follow that in K₂SO₄, but in the 10 cm. angles it tended mainly to follow that in MgSO₄.

October 11, 1930.

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2) Loc. cit.

On the Number of Ganglion Cells and Nerve Fibers in Some of the Ventral Nerve Cords of the Earthworm.

2. The Number of Nerve Fibers.

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(With Pl XXIV and 6 text-figures)

INTRODUCTION

The present paper is the second of a series of my contributions on the number of ganglion cells and nerve fibers of the earthworm, and intends to report the numerical relation of ganglion cells and nerve fibers in those ganglia under consideration. Hence in the present work the nerve fibers were chiefly enumerated, especially those passing through the ventral nerve cord or peripheral nerve trunks leaving the ganglia, the cells of which I have already numerically treated. For this purpose it is necessary to learn at first the inner minute structure of the three paired nerve trunks given off from each ganglion as well as that of the ventral nerve cord. So the histological observation was made upon peripheral nerves and ventral cord before actual calculation of nerve fibers contained in them was attempted.

Hitherto, the numerical relation between the ganglion cells and nerve fibers has been established by various investigators, but almost all of them employed, as the materials, vertebrata, such as frog, albino-rat, rabbit, man etc., but not invertebrata, to my regret. In order to ascertain, whether or not what was found in the vertebrata is also true with the nervous system of the invertebrata, I have used chiefly several earthworms and in addition Hirudo nipponica for the purpose of comparison.

MATERIALS AND METHODS

Coinciding with my previous work, I have used three species of Pheretima, P. communissima, (Goto & Hatai), P. hilgendorfi,
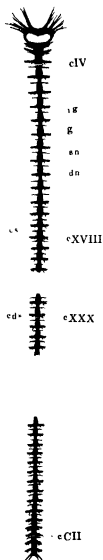
MICHAELSEN, and *P. vittata*, (GOTO & HATAI),
and in addition I have employed two other worms;
Allolobophora foetida, (SAV.), and *Hirudo nip-
ponica*, WHITE, for the purpose of comparison.

These earthworms were collected chiefly in
Sendai and its vicinity during the breeding season:
July, Aug. and Sept.

In order to avoid an abnormal contraction of
the body, the worms were narcotized for a few
minutes in a solution of 0.1 percent chloretone
and were slit along the dorsomedian line with
scissors, and the viscera were removed as much
as possible without injuring the nerves, while
under the influence of anaesthetics. The shrinking
of the body in the fixing solution was avoided
by pinning with wooden pins, and by plunging
it immediately in one of the following fluids:

	Fixing fluids	Fixing time.
1)	LAVDOWSKY's solution	24 hours
	acetic acid 1% 500 cc.	
	potassium bichromate 20 gr.	
	sublimate (saturated) 10 cc.	
2)	Acetic sublimate	30 minutes
3)	ZENKER's solution	24 hours.

Of these fluids, the LAVDOWSKY's solution gave
the best results for this study. After fixation
they were dehydrated and preserved in the usual
manner, then two desirable pieces of the ventral
cord with the body wall were cut off; that is,
one piece from the XVIIth to XIXth and an
other from XXIXth to XXXIInd segment.

Labels on figure: cIV, ig, g, sn, dn, cXVIII, cd, cXXX, cCII

Text-fig. 1. A diagram of the larger nerves of the earthworm in dorsal view,
showing the parts examined. cIV, cutting the interganglion in the IVth segment;
cXVIII, in the XVIIIth segment, cXXX, in the XXXth segment; cCII, in the CIInd
segment, cd, cutting the double nerve; dn, double nerve; cs, cutting the single nerve;
g, ganglion, ig, interganglion; sn, single nerve.

Text-fig. 1 is a diagram of the larger nerves of the earthworm, showing the parts (marked with c) examined in my present work.

To ascertain the number of nerve fibers, it is most necessary to have thin and exact cross section; so the materials embedded in paraffin were cut to 6 or 8 micra. Many stains were used so as to bring out the cross section of the nerve fibers clearly.

  1) DELAFIELD's haematoxylin-eosin on sublimate materials.
  2) HEIDENHAIN's iron haematoxylin on sublimate or bichromate materials.
  3) MALLORY's staining, as an excellent method to differentiate entire tissues for the materials treated by ZENKER's solution.

For the first place, as the most convenient region, I have selected the interganglia (Text-fig. 1, ig.) of the ventral cord, or the portion connecting one ganglion with the next, where the nerve fibers pass through without confusion, for the second, the peripheral nerve trunks were cut at the most approximate regions to the ventral cord, where the nerve fibers do not ramify (See Text-fig. 1).

In these preparations, we can distinguish two kinds of nerve fibers, motor or efferent and sensory or afferent fibers. as the diameters of the former fibers are large and appear in clear outline (Text-fig. 2), containing a single neurofibril, while those of the latter are too small to be calculated. Such structural differences of the two kinds of fibers just alluded to have been well established by many authors, such as, LENHOSSEK ('92), RETZIUS ('00), etc. and are accepted by all subsequent writers.

In practice, I have enumerated only the motor nerve fibers, because they are thought to be concerned mainly with the ganglion cells in the ventral cord, while the sensory fibers are considered to originate from the neuroepithelial cells in the epidermis (LENHOSSEK '92, RETZIUS '00).

The calculation was made by the following method; all the individual fibers contained in the desirable portions were first drawn by means of a "Zeichenprisma" under oil immersion Oc. 4, Ob. 12/1, and then were counted from the drawings by a self-counter to which the needle was attached.

Throughout the entire study, I have used only mature worms of nearly the same size, weight, etc. Moreover, 3-5 individuals were used for each series of determination.

HISTOLOGICAL STRUCTURE OF THE NERVE FIBERS.

Before entering into the calculation of nerve fibers, it will be necessary to describe briefly the histological aspect of the ventral cord and of the peripheral nerve trunks.

1) The ventral nerve cord.

The ventral cord of the earthworm consists of numerous ganglia, which are segmentally arranged, and the interganglia (named by HESS " Connective ") which connect the ganglia (See Text-fig. 1).

It is covered externally by the peritoneum, under which is a layer of longitudinal muscles, together with a sheet of cuticular materials; by these three elements just stated the sheath of the ventral cord is formed. Surrounded by the sheath is found the neuropile, which is divided into two distinct lateral strands, and the median small bundle (Plat. XXIV, Fig. 1, n, and mb.). The former two strands are considered to pass through the entire length of the ventral cord, while the latter median bundle, which is called by FRIEDLÄNDER ('88) " Medianer Nerv", appears in the interganglion only. In each ganglion the ganglion cells are found around the outside of the neuropile.

The neuropile in the ganglion consists of interlacing nerve fibers of various sizes, of efferent and afferent nature, while in the interganglion all nerve fibers take a course nearly parallel to the longitudinal axis, and hence I have only employed the latter for the purpose of calculation in neuropile. The neuropile of each side has three bundles, named by FRIEDLÄNDER ('88) Hauptfaserzügen, which consist of Dorsal-, Lateral-, and Ventralhauptfaserzug (See Plat. XXIV, Fig. 1).

Minute observation of the neuropile reveals that this contains three sorts of nerve fibers of different sizes, namely, large, small and extremely fine. As to the property of these fibers, I have followed, as was stated already, the definition of LENHOSSEK, RETZIUS, etc., that the large and small fibers are efferent in nature originating from ganglion cells in the ventral cord, while the extremely fine fibers are afferent fibers arising from the epidermal layer.

2) The peripheral nerve trunks.

From each ganglion there arise three pairs of peripheral nerves (Text-fig. 1, sn. dn.). The first pair is called the single nerve (named by HESS anterior nerve trunk); the second and third together are

called the double nerve, as they are fused at their bases to a common root, namely anterior and posterior nerve trunks (in HESS' nomenculature middle and posterior). Since these nerve trunks ramify into many branches soon after they leave the ventral nerve cord, I have studied only the most proximal portion of the nerve trunks by cross section, before any branching occurs (Text-fig. 1, c).

The single nerve is composed of both the efferent and afferent fibers and lacks blood vessels, etc. (See Text-fig. 2, B).
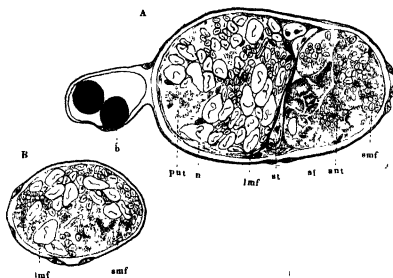
Text-fig 2 Cross section of the single and double nerves in the XXXth segment of the earthworm, P. communissima, ×480 A, double nerve; ant, anterior nerve trunk; B, single nerve; b, blood vessel; lmf, large motor (efferent) nerve fiber; n, neurofibril; pnt, posterior nerve trunk; sf, sensory (afferent) nerve fiber; smf, small motor (efferent) nerve fiber; st, structures of cellular appearance yet unidentified.

As Text-fig. 2, A shows, the anterior trunk of the double nerve is mainly used as the pathway for afferent fibers, because in them only a few of the smaller efferent fibers, while the posterior trunk is almost entirely occupied by the large efferent fibers and afferent fibers occupy small area. These facts have been already ascertained by many authors, such as LENHOSSEK ('92), RETZIUS ('00), etc. These

two nerve trunks are covered by a common sheath accompanying the blood vessels; they have furthermore three elongated structures of cellular appearance yet unidentified in the border-region between the anterior and posterior trunks (Text-fig. 2, A, st) and also a few ganglion cells are present in the distal portion of the nerve trunks.

These unidentified structures appear to hold nuclear-like bodies as well as granular and fibrillar substances, in them but are different from regular ganglion cells, and are constantly present in double nerve. In the affinity to staining agents they differ from the regular nerve fibers, since in MALLORY's staining all other nerve fibers are coloured to blue, while these unidentified structures take a lighter red colour, not only in the nuclear-like body but also in the other substances already mentioned.

So far as I am aware, there are no statements given in regard to this structure and I am unable to determine its nature.

THE RESULTS OF THE CALCULATION

The numerical values in the tables mean always the average obtained from 3 to 5 specimens of each species or variety.

1) The XXXth segment of three species.

Tables I and III show the number of efferent fibers in the XXXth segment, in which none of the special organs besides the alimentary tract, blood vessels and nephridia is found, and for the sake of convenience I shall call such segments "typical segments".

a) Neuropile.

The number of nerve fibers contained in the right and left neuropiles are given separately in order to indicate the symmetrical distribution in both sides, together with the sum of the two numbers.

From Table I, it is clearly seen that in the interganglion of the XXXth segment the numbers of efferent fibers are nearly equal in three different species; *P. communissima, P. hilgendorfi, P. vittata*, being on the average 2427, 2408 and 2069 respectively.

A similar relation holds in the other typical segments XL, LX and LXX (Table II), in which none of the special organs is found, as in the XXX segment, that is, all ganglia belonging to these segments just mentioned gave almost equal numbers of efferent fibers.

TABLE I.

On the ventral cord of XXXth segment.

Specimens	No. of efferent fibers in neuropile				No. of ganglion cells		
	Right half	Median	Left half	Sum	Right half	Left half	Sum
P. communissima	1208	56	1163	2427	608	600	1208
P hilgendorfi	1187	57	1164	2408	580	591	1171
P. vittata	995	55	1019	2069	576	565	1141
P. vittata (prostate in both sides)	1124	54	1085	2263	567	548	1115
P. vittata (prostate in right side)	1094	50	1118	2262	573	567	1140
P. vittata (prostate in left side)	1023	53	1031	2107	553	550	1103

TABLE II.

On P. vittata (without prostate gland).

No of segment	No. of efferent fibers in the neuropile				No. of ganglion cells		
	Right half	Median	Left half	Sum	Right half	Left half	Sum
XXX	995	55	1019	2069	569	533	1102
XL	1017	54	1084	2155	550	541	1091
LX	1009	48	1105	2162	560	565	1125
LXX	1020	50	998	2068	520	564	1084

On comparing these results obtained from the nerve fibers with the number of ganglion cells in corresponding segments, as is shown in Table I, it is clear that the number of efferent fibers in the neuropile is about twice as many as the number given by the ganglion cells. This relation just stated seems to suggest that the given inter-ganglion contains not only those efferent fibers which originate from each corresponding ganglion, but also those coming from the ganglia situated in other neighbouring segments. Since each ganglion is considered to contain besides the unipolar ganglion cells, which make the majority of cell elements, also the bipolar ganglion cells, both

processes of which may enter into both anterior and posterior inter-
ganglia, and, in addition, the nerve fibers may give branching. We
may anticipate under such circumstance, the excess of the fibers over
the cell numbers.

In fact it was actually observed by CERFONTINE, RETZIUS, HALLER,
etc. with GOLGI's method that the processes of the bipolar ganglion
cells pass over 2 or 3 anterior and posterior ganglia in the consecutive
segments.

I am unable to state at this moment how far the efferent fibers
overpass the neighbouring ganglia forwards and backwards.

In short, (1) the number of the efferent fibers as well as that of
the ganglion cells found in these typical segments are nearly the same
in three distinctly different species; and (2) the number of efferent
fibers are greater than that of the ganglion cells in all cases.

I attempted to ascertain the number of efferent fibers in the
neuropile of *Allolobophora foetida* and of Hirudo nipponica, but failed
on account of the extreme fineness of the nerve fibers to be enumerated.

b) Peripheral nerve trunks.

From Text-fig. 2 and 3, one can find that the diameters of efferent
fibers vary considerably, and in Table II, I have given those large
fibers having a diameter of more than 0.025 mm. (In Table III the
numbers in brackets represent these fibers).

In all cases (From Table III), the numbers of large fibers (in
bracket of Table II) are nearly equal in the single nerve but those
of the smaller efferent fibers show a greater tendency to variability.
Furthermore, it is evident from Table III that in general the single
nerves contain approximately the same number of efferent fibers in
all species of *Pheretima*.

Contrary to the single nerve, the anterior trunk of the double
nerve consists chiefly of sensory fibers, giving such small numbers of
small efferent fibers; as 62, 68 and 74 etc. (See Table III, Column
b and b').

On the other hand, the posterior trunk of the double nerve consists
mainly of large efferent fibers, as with the single nerve, though the
numbers are small, being namely 32, 31 and, 30. The diameter of
these large fibers are also nearly the same in all the three species,
as Text-fig. 3 and 4 indicate, though the largest diameters given by

TABLE III.

Number of efferent fibers in the nerve trunks of the XXXth segment.

Specimens	Single nerve.			Double nerve.					Total sum
				Anterior t.		Posterior t.			
	Right	Left	Sum	Right	Left	Right	Left	Sum	
	a	a'	A	b	b'	c	c'	B	
P. communissima	70 (32)	68 (90)	138	62	64	64 (32)	63 (90)	253	391
P. hilgendorfi	62 (24)	65 (25)	127	68	67	66 (28)	64 (29)	262	389
P. vittata	62 (24)	67 (22)	129	66	69	68 (29)	65 (30)	268	397
P. vittata (prostate in both sides)	61 (23)	65 (23)	126	68	74	63 (31)	67 (32)	272	398
P. vittata (prostate in right side)	68 (22)	66 (23)	134	68	63	64 (30)	63 (28)	258	392
P. vittata (prostate in left side)	68 (28)	71 (24)	139	65	64	62 (31)	65 (29)	256	395

P. hilgendorfi and P. vittata are greater (17 micra) than P. communissima (12 micra).

On other species;

a) Allolobophora foetida.

From the sake of comparison I have examined a few typical segments (XLII, L, and LX) of Allolobophora foetida.

The diameters of efferent fibers in the peripheral nerve trunks are much smaller than those of Pheretima (See Text-fig. 4, E, F). I have given in the following table the total number of efferent fibers found together with the ganglion cells in the corresponding ventral cord.

From Tables III and IV, the ratios between the number of ganglion cells and of efferent fibers in Allolobophora foetida are given, contrasted with the similar values obtained from P. communissima:

	No. of cells		No. of fibers		Ratio
P. communissima	1208	:	391	=	3.1
A. foetida	737	:	216	=	3.4

TABLE IV.

On *Allolobophora foetida.*

No of segment	No of efferent fibers.						No of ganglion cells		
	Single nerve		Double nerve						
			Anterior trunk		Posterior trunk		Sum		
	Right	Left	Right	Left	Right	Left		Right	Left	Sum
XLII	38	37	23	22	49	48	217	368	374	742
L	40	39	24	25	46	44	218	380	372	752
LX	37	39	23	24	45	47	215	361	358	719
Average	38	38	23	24	47	46	216	369	368	737

From this result it is clear that these two species of earthworms, belonging to two different families, give nearly identical ratios. What is meant by this unique coincidence, I am unable to interpret.

    b) *Hirudo nipponica.*

    In order to observe these relations on worms of a different order I have chosen as an example *Hirudo nipponica.* The cross sections of the two peripheral nerve trunks leaving the ganglion of this Annelida are drawn in Text-fig. 4, H and G, which present several differences in the inner structure from those presented by the peripheral nerve trunks of the earthworms. As I have shown above (Text-fig. 4, H and G), some of the efferent fibers of *Hirudo nipponica* possess two or three neurofibrils within each nerve, contrasted with always only one neurofibril in *Pheretima.*

    The sum of the number of efferent fibers in both the anterior and posterior nerve trunks of *H. nipponica* (See Table V) is much smaller than that given by the sum of three trunks of *A. foetida,* whereas the number of fibers in each trunk of the former comes very near to that of the fibers contained in each of the three trunks of the latter (the former being 28, 45, and the latter 38, 24, 46). At

the same time, it is also noticeable that in *H. nipponica* the ganglion cells of the corresponding ganglia are considerably smaller than those in *A. foetida* (the former being 483, and the latter 737).

TABLE V.

On *Hirudo nipponica*.

No. of Segment	No. of efferent fibers				No. of ganglion cells		
	Anterior trunk		Posterior trunk				
	Right	Left	Right	Left	Sum	Right	Left	Sum
LX	27	28	46	44	145	245	240	485
XV	28	26	43	45	142	239	241	480
Average	28	27	44	45	144	242	241	483

If we compare the ratio of ganglion cells to efferent fibers, both animals give nearly equal ratios.

	No. of cells		No. of fibers		Ratio
A. foetida	737	:	216	=	3.4
H. nipponica	483	:	144	=	3.4

It appears as though this particular kind of ratio undergoes no great variation, not only between different species belonging to the same genus, but even when the animals belonging to different orders are compared. However, how extensively this statement applies will be an interesting point to determine.

2) The XVIIIth segment of the three species of *Pheretima*.

As has been stated in my first paper ('28), the prostate gland is invariably found in *P. communissima*, while it is occasionally found in both *P. hilgendorfi* and *P. vittata*, though these two latter species normally lack it. Accordingly, in the XVIIIth segment the number of ganglion cells varies remarkably, whether the given species possesses the prostate gland or not: for instance, in *P. communissima*, which possesses the prostate gland in both sides, there are about 4000 ganglion cells and in *P. vittata*, in which the prostate glands are present, there are 3500, while the same species, when it does not

possess them, gives only 1500 or nearly the same number given by
P. hilgendorfi which is also destitute of its organ.

To show how the number of efferent nerve fibers is affected by
the presence or absence of the prostate gland Tables VI, VII, and X
are presented.

TABLE VI.

On the ventral cord of XVIIIth segment.

Specimens	No. of efferent fibers in neuropile				No. of ganglion cells.		
	Right half	Median	Left half	Sum	Right half	Left half	Sum
P. communissima (prostate in both sides)	1515	65	1502	3082	2021	2029	4050
P. vittata (prostate in both sides)	1494	70	1510	3074	1707	1726	3433
P. vittata (prostate in right side)	1509	62	1282	2853	1522	1396	2918
P. vittata (prostate in left side)	1173	57	1505	2735	1334	1436	2770
P. vittata (without prostate)	1231	60	1196	2487	793	769	1532
P. hilegndorfi (without prostate)	1253	66	1264	2583	758	761	1519

a) Neuropile in the ventral cord.

In the neuropile of *P. communissima* the number of efferent
fibers is as much as 3082, while that of the ganglion cells is 4050.
On the other hand, those two other species; *P. hilgendorfi* and *P.
vittata*, in which the prostate glands are not found, give respectively
2683 and 2487 fibers and 1519 and 1532 cells. Now if we compare
the number of efferent fibers with that of ganglion cells, we notice at
once that in *P. communissima* the number of cells exceeds that of
the fibers, while in the other species, which does' not possess the
prostate gland, the contrary relation is shown, giving an excess of
cells over the number of fibers (See Table VI).

These relations just stated may also be seen from Chart 1, in
which the numbers of ganglion cells in the corresponding ganglia are

given with the numbers of efferent fibers, estimated in the neuropiles and peripheral nerve trunks.
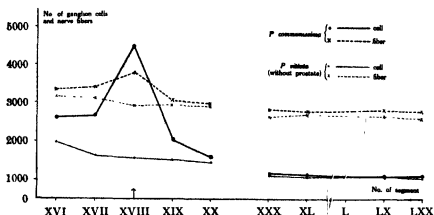
Chart 1. Showing the relation between the number of ganglion cells and efferent fibers in the neighbourhood of the XVIIIth and the XXXth segments of two species of Pheretima, one of which (P communissima) possesses the prostate gland in both sides, and the other of which (P vittata) lacks it (based on Table VII, and Chart 1 of my previous work)

If we compare the two species of worms with respect to the number of cells and fibers, one which possesses the prostate gland and the other which is destitute of this organ, we notice a greater number of cells than of fibers:

	P. communissima		P. hilgendorfi	difference	
Ganlion cells	4050	–	1519	=	2531
Efferent fibers	3831	–	2997	=	834
(in the neuropiles and peripheral nerve trunks)					

We find that the number of ganglion cells shows an excess of 2531, while the excess shown by the fibers is 834. In other words, in the worms with the prostate gland the increase of cell elements is far greater than that of the fibers.

Moreover, I found in P. communissima that these excesses of efferent fibers seem limited in this XVIIIth segment, unlike the number of ganglion cells which shows a noticeable increase in the neighbouring ganglia; for instance, the number of them in the XVIIth

is 2462 cells and 3388 fibers, and in the XIXth 2072 cells and 3048
fibers (See Table VII), while in *P. vittata*, in which prostates are
lacking, the number of cells in these segments is nearly constant.

TABLE VII.

The comparison of the number of efferent fibers of
P. communissima (with prostate) and *P. vittata*
(without prostate).

No of segment	No of efferent fibers of *P. communissima*			No of efferent fibers of *P. vittata*.		
	Both neuropiles	3 paired trunks	Sum	Both neuropiles	3 paired trunks	Sum
XVI	2785	543	3328	2607	553	3160
XVII	2827	561	3388	2561	541	3102
XVIII	3082	749	3831	2487	429	2916
XIX	2622	426	3048	2494	437	2931
XX	2504	398	2902	2473	422	2895

In spite of the comparatively smaller excess of the efferent fibers
in the neuropile in XVIIIth segment, a more remarkable change is
found in the larger peripheral nerve trunks.

b) Peripheral nerve trunks.

In Text-figs. 3 and 4, we may at first recognize the difference
of the size between the nerve trunks belonging to the specimens
possessing the prostate gland and those lacking it. In order to see
the difference more precisely, the areas of the cross sections of the
nerve trunks were measured with a planimeter, and the results show
(Table VIII) that while the single nerves of the specimens with
prostate gland (*P. communissima*) and a variety of *P. vittata*) give an
area of about 20 sq. cm. in ×800 magnification, those without it
give only 11 sq. cm in ×800. Similarly, while in the double nerve
the area of the cross section of the nerve associated with the gland
measures 32 sq. cm. (anterior) and 40 sq. cm. (posterior), the specimens
without the gland measure 23.6 sq. cm. (anterior) and 15 sq. cm.
(posterior).
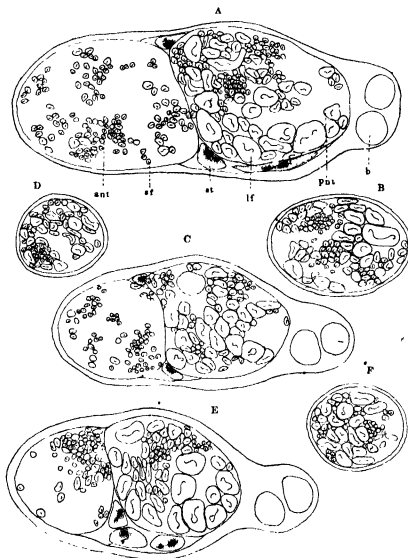
Text-fig. 3. The comparison of the single and double nerves between two segments of earthworms, one of which has the prostate gland and the other not, showing mainly the efferent fibers in the cross sections, ×480. A, double nerve; and B, single nerve in the XVIIIth segment (with prostate gland) of *P. vittata*. C, double nerve; and D, single nerve in the XVIIIth segment (without prostate gland) of *P. vittata*. E, double nerve, and F, single nerve in the XVIIIth segment (without prostate gland) of *P. hilgendorfi*. ant, anterior nerve trunk, b, blood vessel; lf, large efferent fiber; pnt, posterior nerve trunk, sf, small efferent fiber; st, structures of cellular appearance yet unidentified
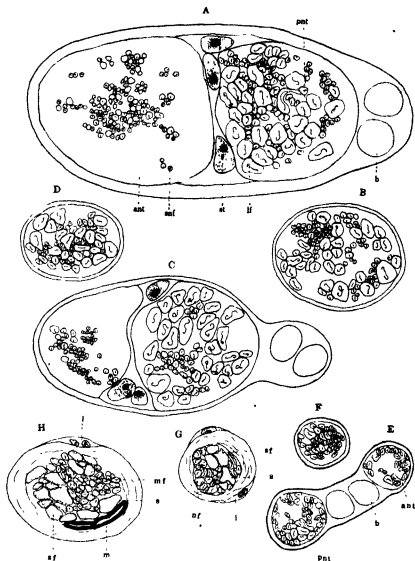
Text-fig 4. The comparison of the single and double nerves between two seg-
ments of earthworms, one of which has the prostate gland and the other not, showing
mainly the efferent fibers in the cross sections, ×480. A, double nerve, and B, single
nerve in the XVIIIth segment (with prostate gland) of *P. communissima.* C, double
nerve, and D, single nerve in the XXXth segment (without prostate gland) of *P.
communissima* (in addition, the nerve trunks of *Allolobophora foetida* and *Hirudo
nipponica* are drawn) E, double nerve, and F, single nerve in the LXth segment of
A. foetida, ×540. G, posterior nerve trunk, and H, anterior nerve trunk in the LXth
segment of *H nipponica,* ×540.   ant, anterior nerve trunk; b, blood vessel; l,
"Lymphspalte"; lf, large efferent fiber; m, muscle; nf, neurofibril; pnt, posterior nerve
trunk; s. "sensorische Schläuche"; sf, sensory nerve fiber; mf, motor nerve fiber; snf,
small efferent fiber; st, structures of cellular appearance yet unidentified.

We further find that there is remarkable change of area especially
on the anterior nerve trunks, when compared with that on the posterior
nerve trunks, and the ratio between these two nerve trunks (See last
Column in Table VIII) gives on *P. communissima* 0.8 and on *P.
hilgendorfi* 1.51; namely, the area of the anterior trunks is more
remarkably influenced by the prostate gland.

TABLE VIII.

Area of sections of nerve trunks.
(in square centimeters, × 800).

Species	Single nerve	Double nerve		Ratio Ant t / Post t
		Anterior t.	Posterior t.	
P. communissima (with prostate) XVIII	19.5	32	40	0.8
P. communissima XXX	11	23.6	15	1 57
P. vittata (with prostate) XVIII	20	34	36	0.94
P. vittata XXX	9.5	23	14 5	1.59
P. vittata (without prostate) XVIII	10	23.6	16	1.47
P. hilgendorfi (without prostate) XVIII	11	23.5	15.5	1.51
P. hilgendorfi XXX	9.5	23	14 5	1.59

Change of the area on cross sections corresponds mainly to the
content of efferent fibers (Table IX), and shows about twice as large
in *P. communissima* and a variety of *P. vittata* which both possess
the prostate gland, as in the others lacking the prostate (Compare
Columns A, B with D, C in Table IX). From Table IX we further
find the following differences in the number of fibers between the
two forms of earthworms, one with prostate and the other without
that gland:

TABLE IX.

Number of efferent fibers in the nerve trunks of the XVIIIth segment.

Species	Single nerve			Double nerve					Total sum
				Anterior t		Prosterior t		Sum	
	Right	Left	Sum	Right	Left	Right	Left		
(A) P communissima (prostate in both sides)	103 (57)	106 (38)	209	146	138	122 (44)	134 (42)	540	749
(B) P vittata (prostate inboth side)	102 (32)	98 (36)	200	131	133	123 (43)	133 (43)	520	720
P vittata (prostate in right side)	91 (32)	61 (20)	152	140	74	96 (35)	64 (32)	374	526
P vittata (prostate in left side)	58 (22)	101 (31)	159	78	140	67 (37)	105 (40)	390	549
(C) P vittata (without prostate)	66 (21)	62 (23)	128	77	75	74 (32)	75 (50)	301	429
(D) P hilgendorfi (without prostate)	65 (20)	62 (21)	127	70	75	70 (32)	72 (33)	287	414

	P communissima (with prostate)		P hilgendorfi (without prostate)		difference
No of fibers of single nerve	103	—	65	=	38
No of fibers of double nerve	146	—	70	=	76

It is also to be noted that the increase of the number of fibers is chiefly contributed to by the smaller fibers, rather than by the large fibers. This increase is shown in Table X, in which we find the increase of 11 large fibers, contrasted with the increase of 38 small fibers.

The fact just mentioned will suggest to us that the efferent fibers which innervate the prostate gland may be mainly smaller ones. In order to make clear that this increase in number of fibers was brought about by the addition of prostate gland in *P. vittata*, Table XI was made, in which the number of ganglion cells is also added for the convenience of comparison.

In Table XI, the greatest increase of efferent fibers (72%) is observed in the double nerve and least in the neuropile (23%). When

TABLE X.

Number of efferent fibers in posterior trunk of double
nerve of the XVIIIth segment.

P vittata	Large fibers	Smalle fibers
With prostate	43	80
Without prostate	32	42
Number of fibers increased	11	38

we compare the total number of efferent fibers with that of ganglion
cells, we are surprised at the relatively smaller increase of the fibers,
contrasted with the increase of cells, that is, 30% of efferent fibers
to 124% of ganglion cells.

TABLE XI.

P vittata	Number of efferent fibers in the XVIIIth segment				Number of ganglion cells
	Neuropile	Single nerve	Double nerve	Sum	
With prostate	3074	200	520	3794	3437
Without prostate	2487	128	301	2916	1532
Number of fibers increased	587	72	219	878	1905
	23%	56%	72%	30%	124%

In short, the increase of efferent fibers caused by the presence of
the prostate gland is not parallel with that of the ganglion cells, or
the number of ganglion cells in the XVIII segment, which surpasses
the total number of efferent fibers found in the neuropile, and the
single, and double nerve together, amounts to one thousand. This
excess of ganglion cells may be accounted for if we assume that not
all cells send their processes outside of the ganglion.

3) The symmetrical relation of the number of efferent fibers as
is shown by the right and left halves of the ganglion.

My previous work showed that, in all the ganglia examined, the
number of ganglion cells is almost equal on both sides In order to

see whether or not the number of efferent fibers is also identical in
both halves, I have calculated these separately. As was anticipated,
the efferent fibers like the ganglion cells exhibited symmetricity in
their numerical relation; namely, the neuropiles as well as the peri-
pheral nerve trunks possessed on both sides nearly the equal number
of efferent fibers (See Table VI and IX), as the following condensed
Table shows:

	Neuropile Right Left	Single nerve. Right Left	Anterior trunk. Right Left	Posterior trunk. Right Left
P. communissima	1515 1502	103 106	146 138	122 134
P. hilgendorfi	1253 1264	65 62	70 75	70 72

In the case of the worms with asymmetrical organization, for in-
stance, in P. vittata having non-paired prostate gland, we find that
there exists considerable difference between the right and left halves
in the neuropile as well as in the nerve trunks. It is also to be
stated that the fibers are not at all increased on the side without
this gland, while a considerable increase is observed on the side
possessing the non-paired organ (Compare the neuropiles of the right
and left side in Text-fig. 5, B). This fact is quite contrary to my
previous finding that under such circumstances the number of ganglion
cells showed approximately a similar increase in both halves, though
the increase in the side with the non-paired gland tends to be slightly
greater, and shows not so considerable a difference as was noted in

TABLE XII.

On P. vittata possessing the prostate gland in the left side.

P. vittata	Number of efferent fibers.					Number of ganglion cells.
	Neuropile	Single n.	Double nerve.		Sum	
			Anterior t.	Posterior t.		
Left side (with prostate)	1505	101	140	105	1851	1436
Right side (without prostate)	1173	58	78	67	1376	1334
Difference	332	43	62	38	475	102

the increase of the fibers.

To illustrate these relations just stated I have prepared Table XII in which the number of efferent fibers in the right and left side of *P. vittata*, which possesses a gland in the left side only, is given; the number of ganglion cells is also added in the table.

The increase of ganglion cells in both halves in the specimen with non-paired gland may be due to the fact that the fibers which innervate the prostate gland originate not only from the ganglion cells in the same side, but also from those in the other side.

In short, the efferent fibers increase only in the side where the gland is attached, in spite of the fact that under such circumstances the ganglion cells increase in both sides (right and left).

4) The comparison of efferent fibers along the entire length of the ventral cord of *P. vittata*.

In order to obtain some idea as to the number of efferent fibers in the neuropile while passing from the anterior to the posterior end of the body, I have calculated besides those already found (XVIIIth, XXXth, XLth, and LXXth segments) the efferent fibers in the IVth segment and ones belonging to the CIInd segment, and the results are shown in Table XIII. The number of ganglion cells is also added in the same table.

TABLE XIII.

Number of ganglion cells and efferent fibers in some ganglia of the ventral cord of *P. vittata*.

No. of segment	No. of efferent fibers in neuropile.				No. of ganglion cells.			Ratio fiber cell
	Right half	Median	Left half	Sum	Right half	Left half	Sum	
IV	1042	0	1048	2090	1108	1058	2166	0.967
XVIII	1117	60	1173	2350	780	694	1474	1.594
XXX	1081	56	1120	2257	566	558	1124	2.008
CII	671	48	687	1400	490	492	982	1.446

Looking at Table XIII, it will be noted that the number of efferent fibers in the neuropile of the IVth segment (anterior end — See Text-fig.
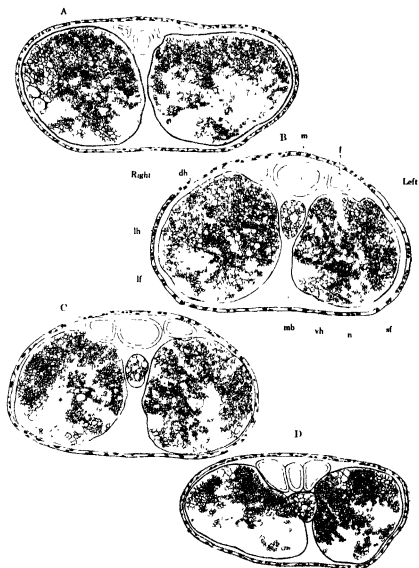
Text-fig. 5   Cross section of the interganglion of the ventral cord in *P. vittata*, showing the difference in the neuropile from head (IVth segment) to tail (CIInd), ×200   A, cross section in the IVth segment. B, cross section in the XVIIIth segment with non-paired prostate gland (the gland existing in the right side only). C, cross section in the XXXth segment; D, cross section in the CIInd segment; dh, dorsal Hauptfaserzug; lf, large motor (efferent) fiber; l, lateral giant nerve fiber; lh, lateral Hauptfaserzug; m, median giant nerve fiber; mb, median bundle; n, neuropile; sf, small motor (efferent) fiber; vh, ventral Hauptfaserzug.

5, A) is less than that found in the other ganglia, while the ganglion cells in the IVth segment indicate a larger numerical value than all the other ganglia under consideration. On the other hand, the CIInd interganglion (posterior end of the ventral cord) gave a lower number of both efferent fibers and ganglion cells than any other ganglia examined (See Text-fig. 5, D).

In order to learn the numerical relation of the efferent fibers and ganglion cells in each segment, Table XIV was made based on the data given in Table XIII.

<p align="center">TABLE XIV.</p>

<p align="center">The numerical comparison of the efferent fibers and
ganglion cells of the ventral cord.</p>

No. of segment of P vittata	No of efferent fibers.	No of ganglion cells	Ratio
IV	2090	2166	0.967
XVIII	2554	1503	1.693
XXX	2257	1119	2.016
CII	1420	982	1.446

It is to be concluded from Table XIV that the ratio, or number of fibers per one ganglion cell, is largest in XXXth (See Text-fig. 5, C), and smallest in the IVth segment which contains the anterior end of ventral nerve cord. This fact may suggest to us that the majority of efferent fibers originating from a ganglion in the more anterior segments tends to proceed towards the posterior direction.

<p align="center">SUMMARY.</p>

1. In the typical segments (XXX, XL, LX, and LXX), where organization is similar and simple, the numbers of efferent fibers in the neuropile and in the three peripheral nerve trunks are approximately equal in the three species of *Pheretima* (*P. communissima* 2818, *P. hilgendorfi* 2797, and *P. vittata* 2456). The number of efferent fibers is more than twice that of the cells in the corresponding

ganglion; the ratio of the efferent fibers to the ganglion cells is in *P. communissima* 2.33, in *P. hilgendorfi* 2.38 and in *P. vittata* 2.15.

From this it seems probable that the efferent fibers of a peripheral nerve trunk and neuropile originate not only from the concerning ganglion, but also from the neighbouring ganglia, and such cells must also exist in the ganglia which issue two or more efferent fibers or bifurcating nerve fibers.

2. As the results of the appearance in XVIIIth segment of the prostate gland the efferent fibers increase considerably more in the peripheral nerve trunks than in the neuropile; that is, an increase of 56% in the single nerve and 72% in the double, while in the neuropile it amounts to only 23%.

A cross section shows that the area of the anterior trunk of the double nerve indicates a greater increase than other nerve trunks. This increase is chiefly due to the greater increase of the area occupied by the generally smaller fibers. In the XVIIIth segment of the worms with the paired prostate gland, the ganglion cells far exceed the number of efferent fibers contained in all the nerves arising from the ganglion.

3. Similarly as has been already found in the ganglion cells, the number of efferent fibers are nearly identical in both (right and left) sides of the ganglion. Such symmetrical distribution of the fibers is found in the neuropile, as well as in the peripheral nerve trunks. When, however, the worm possesses a non-paired prostate gland, the number of efferent fibers shows a greater increase in the corresponding half, or asymmetrical distribution of the fibers in both halves in the neuropile as well as in the peripheral nerve trunks, while under the same circumstances approximately the same number of ganglion cells is found in both halves (OGAWA '28).

This is interpreted to mean that the fibers which innervate the prostate gland originate not only from the ganglion cells of the side where the prostate is found, but also from those of the other side.

4. In *Allolobophora foetida* and in *Hirudo nippenica* it was found that the number of ganglion cells in the ventral nerve cord and that of efferent fibers in the peripheral nerve trunks are very much smaller than in any one of the three species of *Pheretima* examined, but the ratio of ganglion cells to efferent fibers is found similar not only

between the worms of different genus, but also between those of a
different order.

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EXPLANATION OF PLATES

PLATE XXIV.

All microphotographs indicate the nerve fibers in the cross section of the
ventral cord, and the peripheral nerve trunks of *P. communissima*, ×240.

Fig 1. Cross section of the ventral cord at the interganglion in the XXXth
        segment (without prostate gland)

Fig. 2. Cross section of the double nerve in the XXXth segment.

Fig. 3. Cross section of the single nerve in the XXXth segment.

Fig. 4. Cross section of the ventral cord at the interganglion in the XVIIIth
        segment (with prostate gland).

Fig 5 Cross section of the double nerve in the XVIIIth segment.

Fig. 6. Cross section of the single nerve in the XVIIIth segment

ABBREVIATIONS

    ant, anterior nerve trunk.
    b, blood vessel
    dh, dorsal Hauptfaserzug
    l, lateral giant nerve fiber
    lh, lateral Hauptfaserzug
    m, median giant nerve fiber.
    mb, median bundle.
    mf, motor (efferent) nerve fiber
    n, neuropile
    nf, neurofibril.
    pnt, posterior nerve trunk
    sf, sensory (afferent) nerve fiber.
    st, structures of cellular appearance as yet unidentified.
    vh, ventral Hauptfaserzug
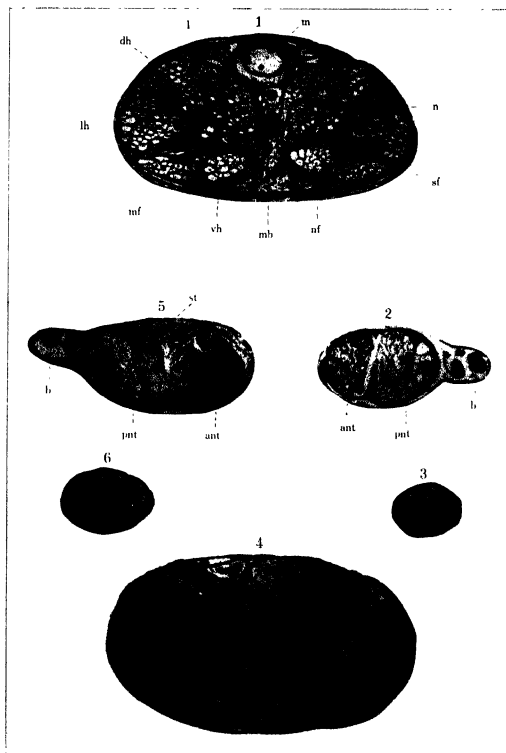
Preliminary Studies on the Physiology of the Pulsating Blood Vessels of the Earthworms

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(With 9 Text figures.)

INTRODUCTION

Since Home (1818) and Duges (1828) reported in regard to the circulation and the blood vascular system of the Annelida, there have been many investigators who have studied the structure of the blood vascular system in Annelida   But up to the present time on the physiology of the pulsating blood vessels few investigators have reported, as far as the writer is aware   A J Carlson (1908 in Nereis and Arenicola), H Stubel (1909, in Lumbricus)   K v Haifner (1927 in Lumbriculus) and H Federighi (1928 in Arenicola) have studied the physiology of the pulsating blood vessels

But facts concerning the physiological properties between the hearts and the pulsating dorsal blood vessel seem to me to be still imperfect and for this reason the writer undertook to study mainly on this point

In regard to the anatomical arrangement of the blood vascular system, K N Bahl's work (1921) furnished much information to the writer

Before going further, the writer wishes to express sincere thanks to Dr S Hatai for his kind direction

MATERIAL AND METHOD

The earthworms used in the present work are only adults of two species in Genus Phretima — Ph megascolidioides (Goto et Hatai) and Ph communissima (Goto et Hatai) — which are the most common earthworms living in Japan   They were collected in Sendai, and, in the vicinity of the city of Imaharu in Shikoku

Of these two species Ph. megascolidioides is very suitable for

the various observations and experiments, as its body is larger than the other species, and its blood vessel are also very large. But there is an inconvenient point in this species, that the observation of the blood vessel through the body wall is difficult on account of its thickness and the large accumulation of pigments in it. *Ph. communissima* is smaller than the former, but it is very favourable for observing the blood vessel through the body wall.

METHOD I.

a. The earthworms were anaesthetized in 9% alcohol for 50-55 minutes at the room temperature of 15-25°C.

To observe the influence of the alcohol the following method was adopted. The earthworms was put into a glass tube, which had been cut longitudinally in half, as shown in Fig. 1, and 9% alcohol was poured into it; then it was covered with a glass plate.

Fig. 1.
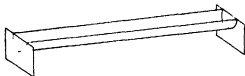
The temperature of the anaesthetic was kept constant at the proper grade by immersing this tube in running water. Then the direction and frequency of the peristaltic waves of the dorsal vessel were observed by the naked eye or by a low power microscope through the glass cover with a stop watch. In various experiments this frequency was also counted from the curve obtained from the photographs which I shall describe separately below.

b. To observe the influence of temperature on the blood vessel, a whole or a part of the body put into a dish which had been filled with water or RINGER's solution. In order to raise or lower the temperature of this dish it was plated into a larger dish which was filled with water of the proper temperature.

c. As the source of the electric current, one dry battery (Nippon dry battery Flate No. 2) was employed. The primary circuit was composed of the battery, make and break key, primary coil of the inductorium, secondary circuit, secondary coil and platinum electrode. The current passing through these circuits was used as the electric stimulus.

d. In order to isolate the brain, the body wall of the head region

was carefully cut open without injuring the dorsal vessel and then the brain was isolated with the pharynx.

To exstirpate the ventral nerve cord, one part of the body wall was carefully incised along the dorsal line so as not to touch the dorsal blood vessel and separating one side of it from intestine as is shown in Fig. 2. A part of the dorsal blood vessel was then dissected out. Then keeping apart the exposed intestine from the ventral side of the body wall, the 4-5 ganglia of the ventral nerve cord was cut with a little and sharp scissors so carefully that the ventral blood vessel was not injured. In all cases only one side of the body wall was opened so that the commisural vessel should not be cut off.
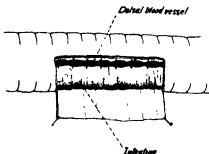
Fig. 2   Dorsal view of operated worm
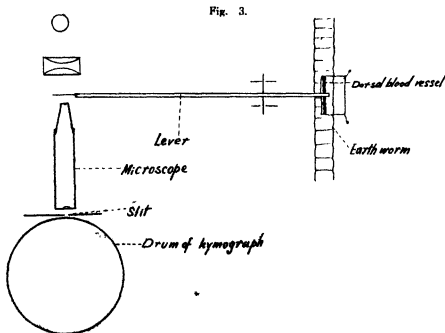
Fig. 3.

METHOD II.   Photographic method of tracing the peristaltic waves of the dorsal vessel.

One end of the lever, which was made of straw, was placed on the dorsal vessel exposed by Method I, explained in the above, and the movement of the other end of the lever was magnified by the microscope and was traced on a photographic film by means of the kymographic method. (Refer to Fig. 3 and 4.)
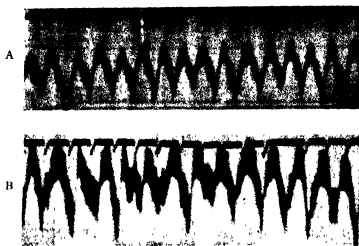
Fig. 4   Photographic tracing of the peristalsis of the dorsal blood vessel under anaesthetization
Upper line - time in five-seconds intervals

THE NORMAL FREQUENCY AND THE DIRECTION OF THE PERISTALSIS OF THE DORSAL BLOOD VESSEL.

It has been described already by CARLSON (1908) that in Arenicola and Nereis this contration wave may, and often does, fade away in the middle region of the heart. In normal conditions in Lumbricus a contraction wave once started in the posterior end of the dorsal vessel traverses the whole length of it in most cases (STÜBEL, 1909). And in Lumbriculus variegatus the dorsal vessel shows higher frequency in the posterior region, and lower in the anterior (HAFFNER, 1926). However in my experiment it was found that within the same individual the frequency of the peristalsis in the posterior part of the dorsal

blood vessel almost agreed with those in the middle and the anterior as is shown in Table 1.

TABLE 1.

Peristalsis number at three parts of the dorsal vessel.

Mater	Temp	Number of Peristalsis per Minute		
		in Anterior	in Middle	in Posterior
Ph. megas.	21°C	12	12	12
,,	21.5	19	18	18
,,	22	15	15	15
,,	22	15	15	15

In the large number of individuals employed in this experiment there were several exceptional ones in which the difference in the number of peristalsis between the posterior and anterior region were 2-3 per minute. From this fact it can be safely said that, in general, the peristaltic waves which start in the posterior end reach the head region without changing this period. This fact agrees with STUBEL's observation on Lumbricus.

Also the number of peristalsis of the hearts generally agrees with the frequency given by the dorsal blood vessel. There are, of course, wide individual variations as to the number of peristalsis even within the same species as will also be seen from Table 2.

TABLE 2.

Peristalsis number of normal earthworms.

Sp.	No. of Worms examined.	Temp.	Average No of Pers. per Min.	Max. & Min.
Ph. megas.	11	20°C-23°C	13	10-18
,,	11	23 -26	14	11-17
Ph. comm	13	23 -26	21	16-28
,,	9	11 -14	10	9-11

According to STÜBEL, the frequency in Lumbricus is 15-20 per minute at room temperature, while HAFFNER counted in Lumbriculus

60–80 per minute in the posterior part of the dorsal vessel, 24–30 per minute in middle and 10–18 per minute in the anterior at 15°C.

INFLUENCE OF ALCOHOL

Although many investigators have often used 5–7% alcohol as an anaesthetic, 9% alcohol was chiefly employed in the present experiment. The reason as follows: the earthworms, when they are anaesthetized for longer periods (70–120 minutes) in lower concentrated alcohol (6%), become very weak and are with difficulty revived, on the other hand when they are anaesthetized for a comparatively short time (45–55 minutes) in higher concentrated alcohol (9%), they become, indeed, also weak, but they seem to recover more rapidly.

The temperature of the anaesthetic is almost equal to the room temperature, i. e. 15–23°C. Although I have tested the influence of the alcohol on the dorsal blood vessel in four different periods of anaesthetizing; that is, 30, 40, 50 and 60 minutes, only the influence of anaesthetization for 50 minutes at 15–18°C will be described in this report. As is shown in Fig. 5, when the earthworm is put into the anaesthetic, the frequency of peristalsis begins gradually to decrease and the amplitude also becomes gradually weak. When, however, such an anaesthetized worm is removed from the alcohol and is placed on the filter paper, moist with ordinary tap water, the number of peristalsis increases gradually and after 25–30 minutes reaches the maximum number. Generally the frequency of the peristalsis at the maximum point is 2–4 per minute, though some cases are given 8 frequencies per minute more than the normal case. This maximum rise is followed by a rapid decrease, giving a far smaller number of peristalsis than the normal. This decrease is again followed by a gradual increase till it reaches the normal frequency. It is generally the case that 15–20 minutes after the anaesthetized worm is brought out from alcohol and is on the moist paper, it begins to move both ends of its body, and consequently all tests were made before such movement of the body appeared.

It was noted that the blood vessel of the worm behaves normally for 7–10 minutes, but after this period the peristaltic wave on the dorsal vessel beggins to show a reverse course distinctively in the
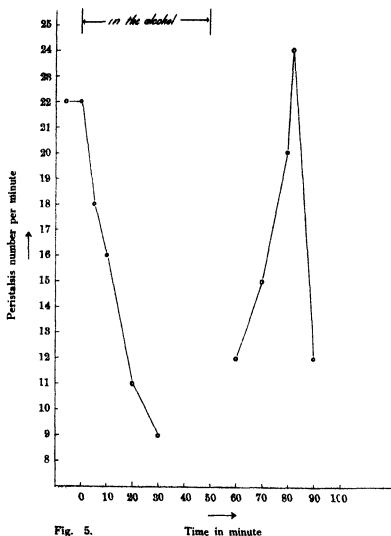
Fig. 5.   Time in minute

posterior region; that is the peristaltic waves divided into two directions, forwardly and backwardly, near the 10th–13th segment from the tail end, while in no other part such changes are seen. But in the next moment in the anterior pre-clitellum segments the dorsal vessel presents the peristaltic waves alternatively progressing forward and backward, while the middle region only keeps a normal direction. Associated

with the irregular directions of peristalsis, the interval between each peristalsis shows wide variation.

After 20 minutes: The backward movement of peristalsis is now seen in the middle of the body instead of between the 10th–13th posterior segments. The interval between each peristalsis becomes more irregular. Every once in a while a stronger wave appears in the head region which propagates the whole length of the dorsal vessel to the tail without interruption.

After 30 minutes: The backward movement of peristalsis starts in the far anterior segments or near the clitellum. In the anterior region the wave propagates mostly backward and occasionaly forward. In the posterior region, the peristalsis becomes very weak and is almost invisible.

After 40 minutes: The peristaltic waves appear only ocassionaly in the anterior segments which propagate mostly backward, while in the posterior region they become invisible altogether.

After 50 minutes. The peristalsis of every part of the dorsal blood vessel becomes very weak and is almost invisible except in the heart proper.

From these observations it seems clear that the reverse course of peristaltic waves begins at first in the tail region and then progresses anteriorly as the anaesthetic effects deepen.

When the earthworms are brought out from the alcohol and put on the moist filter paper or a wooden board, it is observed that the first peristaltic wave appears in the head region and propagates backwardly and then the empty posterior vessel is refilled with the blood. Subsequently the peristalsis is established again on the whole length of the dorsal blood vessel, but its direction and frequency are still irregular. To say this more exactly, the forward movement of the peristalsis ceases and then backward peristalsis makes its way at any region, so that the movement of the peristalsis takes exactly different directions alternatively intervened with each other and consequently the interval of every peristaltic wave is very irregular. However the worms almost recover after 8–10 minutes from the abnormality of strength of the peristaltic waves and the interval between them and the normal peristalsis is fully established.

In the worms in the course of recovery it is often observed by

means of the low power microscope that a large peristaltic wave is
made of a number of very small waves, as will be seen from photo-
graphic tracing Fig. 6.

Fig 6 Photographic tracing
of the peristalsis

If the temperature of the anaesthetic
is made too high (26–30 C), the blood
stagnates in both the hearts and the dorsal
vessel near the clitellum. That portion of
the body where the vessel is filled with
stagnated blood becomes swollen and
exhibits an appearance of water-blisters
producing empty blood vessels in the rest.

INFLUENCE OF TEMPERATURE ON THE PERISTALSIS

The influence of the temperature upon the peristalsis of the earth-
worms is very strong as I have shown in Table 3.

In all the subsequent experiments the temperature was raised from
12°C to 41°C in about 22·23 minutes and was lowered from 13°C
to 0°C in about 17 minutes.

Table 3 shows that the rate of peristalsis of the dorsal blood
vessel was augmented with the rising temperature up till 32 C was
reached, where the maximal rate of peristalsis is recorded. This
maximal point is followed rapidly by decreasing peristalsis propotional
to the further rise of temperature till it disappears at 40 C (Refer to
the Fig. 7).

TABLE 3.

Number of peristalsis influenced by temperature.
(Number was counted at the middle point of body.)

No. of Exper. Temp.	Peris. Number per Min.				
	I.	II.	III.	IV.	V.
11.5					
12.0		10		9	12
12.5	11		11		
16.0					18

No. of Exper.	Peris. Number per Min.				
Temp.	I.	II	III.	IV	V.
16.5				18	
17.0	17	16			
17.5			18		
20.0	30				
21.5				24	
22.0		24			23
22.5	40		23		
25.0	56				
26.0					33
26.5				36	
27.0		30			
27.5			36		
28.0					45
32.0		45		45	
32.5	72		55		51
36.5				36	
37.0	60	23			20
37.5			36		
39.5			30		
40.0	15	0			
40.5			0		
41.0				0	
41.5	0				

TABLE 4.

Number of peristalsis influenced by temperature.
(Number was counted at the middle point of body.)

No. of Exper.	Peris. Number per Min.			
Temp.	I.	II.	III.	IV.
14.2	10			
13.0		11	11	
12.1				10
9.0	8	10		9
8.0			8	
5.0	6	6		
4.0			5	5
2.5	5			
1.0		2		3
0.0	0		0	
-0.5		0		0

The graphic presentations based on the data given in Tables 3 & 4 are shown in Fig. 7 & 8 respectively.

The direction of the peristaltic waves is normal before the temperature reaches 32°C, although the frequency of the peristalsis accelerates very rapidly. Beyond 32°C the peristalsis becomes gradually very weak, and its direction becomes very irregular and above 39°C one fails to observe with the naked eye any peristalsis. The earthworms invariably die at a temperature above 40°C.

The data on the influence of lower temperature upon the peristalsis is collected in table 4. As is seen in Table 4, the frequency diminishes gradually as the temperature falls. Below 5°C not only the recording of the exact number of peristalsis is very difficult, but the direction
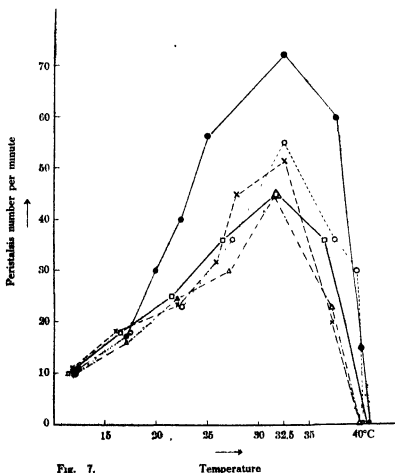
Fig. 7.   Temperature
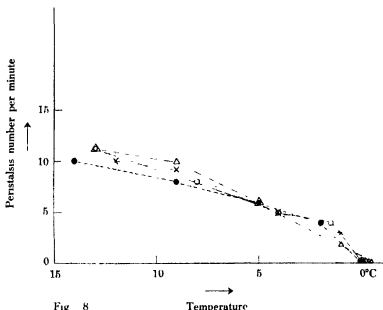
Fig 8   Temperature

as well as the interval of peristaltic waves become irregular. At the
temperature of about 0°C the peristalsis almost ceases (Refer to the
Fig. 8.)

Influence of Temperature on the Parts of the Body.

STÜBEL either warmed or cooled the small portion of the blood
vessel directly and said, "Lokale Kälteeinwirkungen scheinen keinen
deutlichen Einfluss auf den Verlauf der Peristaltik zu haben."

However in the present experiment, as is shown in Tables 5, 6, 7
and 8, when the body anterior to the clitellum is warmed, not only
the rate of the peristalsis of the dorsal blood vessel and of the hearts
in this region increases, but the tail region also shows some increase.
But cases are noted in which the rate of peristalsis shows initial
increase with the quick rise but diminishes within 2–3 minutes and
returns to the normal value.

Warming the tail region, the resulting phenomena are almost the
same as in the former case.

On the contrary when the part anterior to the clitellum is cooled,
the rate of the peristalsis of the hearts and the dorsal vessel in this

region decreases with the falling of the temperature, and decrease is noted in the tail region.

TABLE 5.

Peristalsis number of the anterior and posterior regions of the dorsal vessel when the anterior portion to the clitellum is warmed.

Time Min	Temp.	Peristalsis number per minute	
		in anterior por.	in posterior por
0	19°C	18	17
2	26	20	20
5	26	18	18
6.5	30	23	21
8	30	18	18
11	30	18	18
15	30	18	18

TABLE 6.

Peristalsis number of the anterior and posterior regions of the dorsal vessel when the tail region is warmed.

Time Min	Temp.	Peristalsis number per minute	
		in anterior por.	in posterior por
0	21°C	17	17
1	26	18	23
4	26	23	23
6	30	24	26
9	30	24	24
12	30	24	24

TABLE 7.

Peristalsis number of the anterior and posterior
regions of the dorsal vessel when the anterior
portion to the clitellum is cooled.

Time Min	Temp.	Peristalsis number per minute	
		in anterior por.	in posterior por.
0	19°C	20	20
2	13.5	13	13
5	13.5	9	11
7	13.5	8	9

TABLE 8.

Peristalsis number of the anterior and posterior
regions of the dorsal vessel when the
tail region is cooled.

Time Min.	Temp	Peristalsis number per minute	
		in anterior por.	in posterior por.
0	19°C	14	15
1	13	12	10
4	13.3	12	10
10	13.8	12	12
14	14	14	14
18	14	14	14

SOME PHYSIOLOGICAL PROPERTIES OF HEARTS
AND DORSAL BLOOD VESSEL.

The following observations were made to discover whether or not
peristalsis of the hearts behave similarly or differently under the varied
conditions from that of the dorsal blood vessel.

Experiment I. A. (Material is anaesthetized.)

If the blood flow into a heart was prevented by fastening its upper
part with a silk thread, we noted that the heart continued peristalsis
faintly, though the direction of the blood flow was too indistinct to
be determined. It was further noted that if the lower part of the

heart was fastened, the identical results were obtained.

Experiment I. B. 1. (Material is not anaesthetized.)

When the middle point of the body was bound with thread, the direction of the peristalsis was disturbed and at first it propagated forward in the proximal half and backward in the distal half with normal frequency. Instead of binding only the middle point of the body, if two points including ten segments were bound, then, in these ten segments between the two bindings, the peristaltic waves progressed forwardly then backwardly. In the anterior of the anterior fastening the peristalsis progressed forwardly while in the posterior of the posterior fastening, it progressed backwardly. But such confusion of the direction of the peristalsis is temporar and the body as a whole soon recovers its normal direction with normal frequencies, including the ten bound segments.

B. 2. (Material is anaesthetized.)

If instead of binding with thread round the body of the worm, two points of the dorsal vessel itself are bound so as to make the blood flowing in the region bound to be intercepted from the general dorsal circulation, then this bound region becomes empty, and shows no peristaltic waves. After a short while this region is refilled gradually with blood by inflow from the commisular vessels which are connected with the ventral blood vessel, and then the peristaltic waves appear from the posterior to the anterior, though their frequency is not regular. Thus the contraction waves within the bound region do not subsist longer and a state of relaxation appears sooner or later producing entire disappearance of peristalsis, though all other parts of the vessels show normal peristalsis.

If two points of the dorsal blood vessel are made to contract strongly with any stimulation, these two points remain contracted for a longer period. While the contraction remains, the vessel behaves the same as in the case of binding of the dorsal vessel.

Experiment II. A. (Material is anaesthetized.)

In both the anterior and posterior part of the dorsal and ventral vessels were bound together at two points, so as to include the hearts and dorsal and ventral vessels in the 6–9 segments, during the first five minutes the peristalsis of the hearts and the dorsal vessel included between the two bindings, were regular and the direction of the

peristalsis also was normal. But later the peristalsis becomes irregular, now forward and then backward in the dorsal vessel and, similarly, now ventralward and then dorsalward in the hearts. The frequency of the peristalsis became very irregular. Such alternations as these just stated were soon followed by feeble pulsation as well as by diminished frequency in both the dorsal blood vessel and the hearts.

B. (Material is anaesthetized.)

When the blood flow of all the hearts was intercepted by electrical stimuli being given to both ends of each heart in quick succession, it was observed that for a short time the peristalsis of the dorsal blood vessel was normal in direction, in frequency and in strength. But this regularity did not continue for a long time, and relaxation was gradually induced on the ventral vessel, and the peristalsis was seen no longer.

Experiment III

The test was made to observe the behavior of an isolated heart or a dorsal vessel in RINGER's solution. The results are given in the following protocols.

A. (Material is anaesthetized.)

One of the hearts was isolated at the place where it was joined to the vessels, with scissors, and was placed into RINGER's solution. Owing to the contraction of both ends the blood usually remains within, and the heart continues to pulsate with fair regularity at the room temperature (16–20°C).

There were few cases in which the isolated heart did not pulsate at the same temperature, though it began when the temperature was raised to 20–24 C. The activity of the isolated heart seems roughly to be temporarily propotional to the temperature of the medium, due probably to slight differences in the physiological state of the heart itself as well as in the RINGER's solution used at different times.

B. (Material is not anaesthetized.)

Although a part of the dorsal vessel which attaches to the oeso-phageal region can easily be isolated, isolation without injury of the vessel on the intestine is very difficult, if not impossible. To obtain at the best the latter preparation, the intestinal wall with the dorsal vessel on it was cut off carefully and each corner of the intestinal wall was pinned on a gum plate, as is shown in Fig. 9. The blood

vessel together with the intestine thus pre-
pared is put into RINGER's solution for
observation. If the temperature of the
RINGER's solution is 16-20°C, the isolated
dorsal vessel exhibits peristaltic waves in a
fairly regular manner. At lower than 16°C
the activity of the vessel becomes very weak,
and often the peristalsis is not seen at all.
Cases were found in which the vessel showed
no peristalsis at 16-20 C, but if the tempera-
ture was raised up to about 24°C, peristalsis
began gradually to appear.

Fig. 9.

Dorsal blood vessel

Intestine wall

The influence of the temperature upon the activity of the isolated
dorsal blood vessel thus prepared was almost the same as had been
found on the isolated heart.
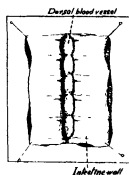
TABLE 9.

Number of peristalsis of isolated heart and dorsal
blood vessel per minute.

No of Exper. Time Min	I		II		III		IV	
	Temp of Ringer's Solution	Peris. No of Heart in 12th Seg.	Temp of Ringer's Solution	Peris. No of dor Vess in 15-20th Seg.	Temp of Ringer's Solution	Peris. No of Heart in 13th Seg.	Temp of Ringer's Solution	Peris. No of Heart in 13th Seg.
0	17°C	23	17 C	—	17°C	weak	17°C	---
5	20	24	19.5	weak	19.5	25	19	weak
10	21.3	21	22.5	weak	22.5	28	21.5	33
15	21.7	19	23	28	23	23	23	36
20	23.2	26	22.5	21	22.5	14	24	26
25	33	26	21.5	irregular	41.5	10	25.5	26
30	22	21	21	16			22	irregular

It was noted that the contraction induced by various kinds of
stimuli so far tested on the hearts and on the dorsal vessel was never
transmitted beyond the immediate locality where the stimulus was
applied, while the contraction waves produced spontaneously, or, in other

words, the contraction induced by the innate stimulus, can propagate.

INFLUENCE OF THE CENTRAL NERVOUS SYSTEM ON THE PERISTALSIS OE THE BLOOD VESSELS.

To determine whether or not the central nervous system controls the peristaltic movement of the hearts and the dorsal blood vessel, I undertook the following experiments. In one experiment, the behavior of the vascular peristalsis was observed after exstirpation of the brain or a part of the ventral nerve cord, and in the other, it was observed

TABLE 10.

Influence of exstirpation of a part of the ventral nerve cord on the number of peristalsis of the dorsal vessel.

Exper No	Materials	No. of Peris. before Exstirp per Min.	No of Peris. after Exstirp. per Min
1	Ph comm	9	8
2	,,	11	11
3	,,	12	13
4	,,	15	15
5	,,	10	10
6	,,	15	15
7	,,	15	15
Average		12	12

TABLE 11.

Influence of exstirpation of the brain on the number of peristalsis of the dorsal vessel.

Exper No.	Materials	No of Peris. before Exstirp. per Min.	No. of Peris. after Exstirp. per Min.
1	Ph. comm.	12	12
2	,,	15	15
3	,,	15	14
4	,,	12	12
5	,,	12	12
6	,,	18	15
7	,,	15	15
8	,,	21	21
Average		15	14

by stimulating the ventral nerve cord of the intact earthworms.

It was found that after the exstirpation of the brain or a part of the ventral nerve cord, the frequency of the peristalsis was identical in 11 cases and higher or lower in 4 cases, as compared with the original number (See Tables 10. & 11.).

While in the case of stimulating the ventral nerve cord the peristalsis of the dorsal vessel was irregularly increased or decreased as is shown in Table 12. But in both cases mentioned above the direction of the peristalsis was not visibly altered either by stimulation or by exstirpation.

TABLE 12.

Peristalsis number of the dorsal vessel influenced by electric stimuli given on the ventral nerve cord.

Exper. No.	Materials	No. of Peris before Stimul. per Min.	No. of Peris. after Stimul. per Min.
1	Ph. megas	4	4
2	,,	9	9
3	,,	9	7
Average		7	7
1	Ph. comm.	11	7
2	,,	14	15
3	,,	14	10
4	,,	5	5
5	,,	11	9
6	,,	9	9
7	,,	11	11
8	,,	21	21
9	,,	10	10
10	,,	24	24
11	,,	13	12
Average		13	12

But according to CARLSON (1908), in Nereis the peristalsis of the dorsal vessel in the intact animal is much more variable than after the exstirpation of the ventral nerve cord and stimulation of the ventral nerve cord with a weak interrupted current exhibits the oesophageal heart in diastol while the rate and strength of the peristalsis of the dorsal vessel is augmented. STÜBEL (1909), on the other hand, reports that the earthworm from which a part of the ventral nerve cord has

been exstirpated, neither exhibits change in the movement of the
dorsal blood vessel, nor in the frequency of the contraction in both
anterior and posterior regions of the operated region as compared
with those given before experimentation. Similarly, that a strong
induction current applied to the ventral nerve cord shows no influence
on the peristalsis of the dorsal blood vessel.

   As to my own observations, given above, I wish to state that the
results need to be further verified with more satisfactory method, as
the technic given in the present report is admittedly imperfect in
photographing the movement of the lever, and I therefore reserve the
final opinion as to the nervous control over the peristalsis of the dorsal
blood vessel to the near future.

                          GENERAL REMARKS.

   The results so far obtained seem to show that the physiological
properties exhibited by the hearts and those exhibited by the dorsal
vessel are almost identical with one possible exception, that the activity
of the former appears to be somewhat stronger than that of the latter.
It was noted in the experiments given in the above that the hearts
and the dorsal vessel may pulsate independently of each other (Ex-
periment III. A & B) and further more that the peristaltic wave does
not originate from the definite center but may originate from any
place on the hearts and the dorsal vessel (Experiments I. II. III.).
What is the impulse which induces the contraction waves on the dorsal
blood vessel and the hearts and propagates normally in one direction
only, can not yet be told from the above experiments. However one
point is clearly established, that the peristaltic waves are to be induced
whenever and whereever the blood is contained within the vessels.
STUBEL (1909) has shown that in the wall of the dorsal vessel and
the hearts are found peripheral nervous plexus which consist of nerve
fibers and cells and they anastomose with each other. It seems there-
fore reasonable to suppose from the facts just given that the peristaltic
waves which are once established by some as yet unknown stimulus
may propagate through the activity of these nervous elements which
lie in the vessel wall.

   The evidences so far obtained in regard to the relation between

the vascular system and the nervous system seem to show an existence of some nervous control over the peristaltic behavior these vessels, but unfortunately the technic empolyed is not entirely free from defects and I therefore reserve this question to the near future.

SUMMARY

1. In the normal earthworms the peristaltic waves once started in the posterior end of the dorsal vessel traverse the whole length of the vessel, while those in the hearts traverse from dorsal to ventral. This normal frequency shows 10–18 per minute at 20–23°C, 11–17 per minute at 23–26°C in *Ph. megascolidioides;* 16–28 per minute at 23–26 C, 9–11 per minute at 11–14°C in *Ph. communissima*. The anterior, middle and posterior parts of the dorsal vessel exhibit almost the same frequency.

2. While under the influence of anaesthetic with 9% alcohol for 50 minutes the frequency of the dorsal vessel and the heart decrease gradually and the direction of the peristaltic waves becomes irregular till they grow too feeble to be noticed with the naked eye. However, when the worm at such state was brought out from the alcohol, the frequency increased gradually to higher than at the normal state. This was followed first by a decrease, then by an increase till it approached the initial number at a room temperature ranging from 15°C to 23°C.

3. The frequency of the peristalsis of the dorsal vessel in the normal earthworm increased or decreased in propotional to the rising or falling of the temperature. The frequency seemed to cease at about 0 C; but below 5 C, the exact number could not be recorded, and it reached the maximum at about 32°C, which was followed by decrease till it ceased at about 40°C.

The direction and the activity of the peristalsis became very irregular and weak beyond 32°C. If the anterior portion alone (proximal to the clitellum) or the posterior portion alone was warmed or cooled, the frequency increased or decreased correspondingly.

4. The dorsal vessel or the heart, if bound at one or two parts, produced a transient confusion on the peristalsis, but it soon recovered the normal frequency and strength.

Even when the blood flow passing through the hearts was prevented by artificially inducing the contraction of the hearts, the dorsal vessel showed peristalsis.

The isolated heart and a piece of the dorsal vessel continued to pulsate fairly regularly in RINGER's solution at 16–20°C.

5. The peristaltic waves do not originate from a definite center, but may arise spontaneously from any part of the heart or the dorsal vessel.

The experiments so far carried out show that functional differentiation of the hearts compared with that of the dorsal vessel can not be noticed.

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Physiological Studies on Drosera.

II. On the Effect of Quinine and Atoxyl on Pepsin.[1]

By

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(With 8 text-figures).

I. INTRODUCTION.

In my former paper (1930) it is reported that, in respect to the optimum acidity, the temperature and the products of digestion, Dro-sera-enzyme closely resembles pepsin. In this connection, to know whether they are identical or not, a study was made, in the first place, on the effect of poisons on the enzymes, just as RONA and his co-workers (1924, '27) and many others have investigated this effect on lipase, esterase, etc. As almost all the experiments on record in regard to the action of poisons on pepsin were carried out long ago and there are only a few, which have been made since RONA, as for instance SMORODINZEW's (1924, '25, '28) and others', these results are not comparable to each other. On this account is it is difficult to use our knowledge of pepsin on this point as a criterion to determine the result on Drosera-enzyme. So these studies on the effect of poisons on pepsin were made in the hope that they may throw some light on this question.

In these experiments, quinine-hydrochloride and atoxyl were used as poison, and the effect of these poisons on pepsin was examined under various conditions.

II. EXPERIMENTAL WORKS.

A) The Method of Experiment.

After several preliminary examinations I adopted the nephelometric method as the most satisfactory one. In a given volumetric flask,

[1] Contributions from the Mt. Hakkôda Botanical Laboratory. No. 5

protein, enzyme and poison were mixed, and then immersed in a water bath; after being warmed for some given minutes, a given quantity of the digestive liquid was taken out, poured in a given volume of cold water as soon as possible and made turbid by the reagent. The degree of turbidity was observed by means of KOBER's nephelometer. Care was taken to let the digestion proceed in such a way that it would allow observation of the digestion even after about one hour.

Though usually the nephelometric method has been applied for researches of the proteolytic enzyme, a few faults, both theoretical and practical, are pointed out by BECHHOLD (1922). Care was given to decreasing these faults as much as possible, so that in my experiments the method proved itself to be very satisfactory and the results were always the same for each definite case.

The enzyme used in the experiments was 0.2% pepsin solution (Pepsine Scales MERCK), and to test the enzyme action generally 0.1% edestin solution containing 0.4% hydrochloric acid was prepared, and to cause turbidity 40% solution of sulfosalicylic acid was used.

The edestin solution and distilled water were respectively put in a flask and warmed in water of 39°C for more than 20 minutes, and a little later a 50 cc. volumetric flask which contained one cc. of pepsin solution and 4 cc. of water was warmed at the same temperature. Then 25 cc. of edestin solution and 20 cc. of water were taken with pipettes into the flask and the mixture was shaken and left in the water bath at 39°C. After 5, 15, 30, 45 and 60 minutes, counting from the time of mixing of each solution, 5 cc. of this digestive liquid was taken out. 15 cc. of cold water was then added to it and it was mixed with 0 5 cc. of 40% sulfosalicylic acid to cause turbidity.

Though the experiments were made under various conditions, the temperature and concentrations of edestin and enzyme solutions were always the same as those above mentioned. Moreover when the solution to be examined contained poison, the solution which contained also the same concentration of it was taken as the standard, in order to eliminate any optical error which might be caused by the presence of poison. Such a muddy solution endured usually 3 5·4 hours without change.

In general an alkaloid, such as quinine, is precipitated by sulfosali-
cylic acid, so that in using this acid it is reasonable to question whether
the poison may be precipitated together with the protein, and thus
cause an error in the observation; but no such effect was found, at
least in the concentrations of quinine-hydrochloride here used.

B) The Preliminary Experiments.

To determine whether the turbidity caused by sulfosalicylic acid
goes parallel with the quantity of protein contained in the solution
or not, I tried the following experiments.

Experiment I.

5 cc. each of 0.05%, 0.025% and 0.0125% edestin solution, of which
the acidity was the same, were poured into 15 cc. of cold water;
then to each of them 0.5 cc. of 40% sulfosalicylic acid was added to
cause turbidity. The turbidity was determined in comparison with
that of 0.05% (the height of liquid was kept at 10 mm.), or of 0.025%
(the height of liquid was kept at 20 mm.) edestin solution.

TABLE I.

No	Standard solution	Concentration of edestin solutions.		
		0.05%	0.025%	0.0125%
1	0.05% edestin solution; the height of the liquid 10 mm.	mm. 10.0	mm. 19.6	mm 42.6
2	0.025% edestin solution, the height of the liquid 20 mm.	9.8	20.0	40.2

The results in (1) fluctuate more than those in (2), so that in
the following experiments 0.025% edestin solution is always taken and
the height of the liquid column in the nephelometer was kept at
20 mm.

Experiment II.

I compared solutions which were digested for 30 or 45 minutes

with quinine-hydrochloride or atoxyl to the standard solution which
did not contain these poisons. (2 and 3). As the control, I used the
solution to be examined and a standard-solution neither of which
contained poisons.

TABLE II.

The order of additions : (Pepsin + Edestin) + Poison.

No	Solution.	Time of observation (minutes)	
		30′	45′
		mm.	mm.
1	Control	24.8	33.1
2	1% Qunine *	25.9	35.6
3	1% Atoxyl *	27.1	38.6

* The per-cent of poison means the concentration of it after the completion
of the mixture of the solutions under question And quinine denotes quinine-
hydrochloride The same remark applies to all following tables.

   Experiment III.

   The solution to be examined which contained poison was compared
to the standard-solution with the same concentration of it.

TABLE III.

No.	Solution	Time of observation. (minutes).	
		30′	45′
		mm.	mm.
1	1% Quinine	23.4	29.8
2	1% Atoxyl	27.3	38.3

   From Table II it may be seen that quinine-hydrochloride accelerates
the digestion, but we may find that this result was rather due to the
standard solution itself which did not contain the poison, as is evidently
shown in Table III.
   When I used the standard solution containing quinine, the digestion

was noticed to be repressed. Therefore in order to be free from any optical error because of quinine-hydrochloride in the observation, it was necessary that the salt be also contained in the standard solution. In the case of atoxyl there was no such effect, but I also mixed it in the standard solution, whenever the solution to be examined contained it. Moreover the standard solution was renewed in each case.

C) The Effect of Quinine.

Some investigators have reported that the addition of quinine-sulfate or hydrochloride in a certain concentration causes the repression of digestion, for example quinine-sulfate in 0.5% (CITTENDEN, 1886), quinine-hydrochloride in 1% (FUJITANI, 1905) and quinine-hydrochloride in 0.20–0.75% and quinine-sulfate in 0.15–0.75% (ASCHER, 1908). On the other hand, LAQUEUR (1906) found that, in 0.001–0.6%, with quinine the digestion was accelerated while in the 0.8% solution no effect was evident. Recently SMORODINZEW (1925) reported that, in 0.000009–0.2% solution, the addition of quinine-hydrochloride, quinine-hydrobromide, quinine-dihydrobromide, quinine-sulfate and of quinine in a free state neither accelerated nor repressed the digestion. As such was the stand of our current knowledge on this question, further studies were in every way desirable. So experiments were made under various conditions to determine the effect of quinine-hydrochloride on the enzyme.

Since the order of addition of enzyme, protein and poison has an effect upon the digestion (OKAHARA, 1930), it was taken into consideration in this study. In this point may be found one of the reasons of difference between almost all the results of foregoing researches and my own. But in the control which did not contain poison in the solution to be examined, in spite of the different order of addition, no effect was noticed.

Experiment IV.

(a) The order of addition.   (Pepsin + Edestin) + Quinine.

The concentration of quinine-hydrochloride was 1%, 0.1% or 0.001%.   (Table IV and Fig. 1).

TABLE   IV.

No.	Solution	Time of observation. (minutes).				
		5′	15′	30′	45′	60′
		mm	mm	mm.	mm.	mm.
1	Control	12.7	17.2	25.0	33.0	43.0
2	1% Quinine	12.7	16.7	23.4	29.9	37.9
3	0.1% ,,	12.5	17.1	24.6	33.5	43.6
4	0.001% ,,	12.9	17.4	25.0	33.2	42.8
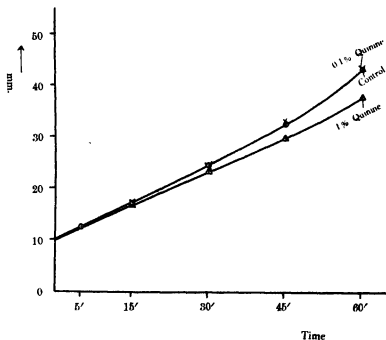
Fig 1. The order of addition. (Pepsin+Edestin)+Quinine.

1% quinine-hydrochloride showed a tendency to repress the diges-tion, while in a weaker concentration than 1% no effect was evident.

   Experiment V.

   (*b*) The order of addition.   (Pepsin + Quinine) + Edestin.

TABLE V.

No	Solution.	Time of observation. (minutes)				
		5′	15′	30′	45′	60′
		mm.	mm.	mm.	mm.	mm.
1	Control	12.4	17.1	25.1	32.5	43.1
2	1% Quinine	12.5	16.7	23.6	29.6	37.7
3	0.1% ,,	12.7	17.2	25.3	33.0	42.8
4	0.001% ,,	12.4	17.0	25.3	32.8	43.0

The result is identical with that of Experiment IV. So it may
be justified to conclude that the order of addition, as between (a)
or (b), has no effect upon the digestion. (Table V and Fig. 2).

Experiment VI.

After the quinine had acted on the pepsin for some given minutes
at 39°C, edestin was mixed with them.

TABLE VI.

No	Solution.	Time of observation. (minutes).				
		5′	15′	30′	45′	60′
		mm	mm	mm.	mm	mm
1	Control	12.4	17.1	25.5	32.5	43.1
2	1% Quinine	12.5	16 7	23.6	29.6	37.7
3	1% ,, (30′)*	10.4	11.6	13.9	16.4	18.9
4	1% ,, (60′)	10.3	11.1	11.6	12.7	13.9
5	1% ,, (80′)	10.3	10.5	11.3	11.8	12.2
6	0.1% ,, (60′)	10.5	11.2	12.3	13.7	15.2
7	0.001% ,, (60′)	12.6	15.7	23.4	29.3	37.0
8	0.00001% ,, (80′)	12.5	16.5	24.2	32.8	43.5
9	Water (80′)	12.4	17.2	25.0	32.4	43.5

*The number in parenthesis shows the time in min., after which the edestin
was added.

When, in 0.001-1% solution, quinine-hydrochloride first acted on
pepsin for 60 minutes, the digestion of edestin afterward added was
repressed. In 0.1-1% solution of quinine-salt, the repression was of
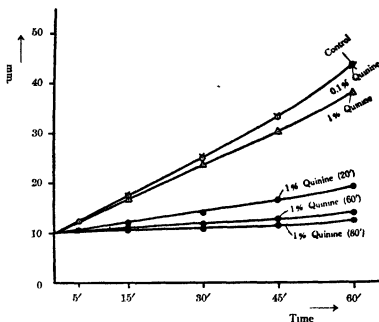
Fig 2   The order of addition. (Pepsin+Quinine)+Edestin.

almost equal grade (Nos. 4 and 6), while in 0.001% it was remarkably
weak and in 0.00001% no more evident. From this result it is
conceivable that the pepsin is attacked by quinine-salt in the proper
dose of the latter, while the protein may protect the pepsin against
decomposition by the quinine-salt. (Table VI and Fig. 2). The next
experiment is in accord with this conception.

     Experiment VII.

  (c) The order of addition. (Quinine + Edestin) + Pepsin.

TABLE VII.

No.	Solution.	Time of observation. (minutes).				
		5′	15′	30′	45′	60′
		mm	mm	mm.	mm.	mm.
1	Control	12.2	16.7	24.4	32.4	43.0
2	1% Quinine	12.3	16.9	24.7	32.6	43.6
3	0.01% ,,	12.2	16.9	24.5	32.8	43.4
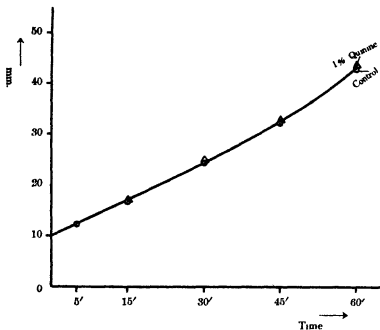
Fig. 3. The order of addition. (Quinine + Edestin) + Pepsin.

In this case there was no effect of the poison on the digestion. (Table VII and Fig. 3).

In Experiment IV, even if the pepsin and edestin were first mixed, these two may not have combined with each other, and the quinine-hydrochloride which was last added may have combined with the pepsin and combatted its action. In other words, the combination of pepsin with quinine is stronger than that of edestin with pepsin. For this reason the same result as from Experiment IV may be produced from Experiment V. But when edestin and quinine are mixed at first and pepsin is not present (Experiment VII), the protein may protect the pepsin from the action of the quinine.

Experiment VIII.

The concentration of quinine-hydrochloride was 0.1% and the quantity of hydrochloric acid in the edestin solution was deminished in a corresponding degree and the acidity of the mixture kept the

same as in the above experiments. Results are given in Table VIII and Fig. 4.

The order of addition. (Pepsin + Quinine) + Edestin.

<div align="center">TABLE VIII.</div>

No	Solution		Time of observation (minutes)				
			5′	15′	30′	45′	60′
1	Control		mm 11.5	mm 15.2	mm 21.6	mm. 27.8	mm 37.2
2	1% Quinine		11.3	14.5	20.2	26.4	33.4
3	1% ,, (80°)*		11.3	14.6	19.0	24.3	29.9

* The number in parenthesis shall be understood in the same sense as in Table VI
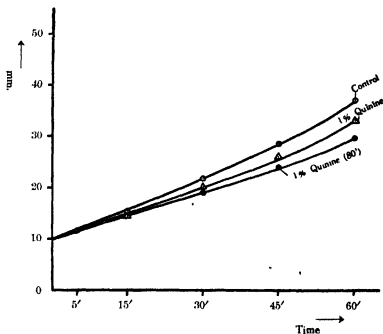
Fig. 4. The same order of addition as in Fig. 2. Quinine acidified.

Even in this case repression occured, though it was weaker than that in Experiment VI. In the form of quinine-salt, the power of repression is weak but not completely destroyed.

D) The Effect of Atoxyl.

The effect of arsenic compounds on pepsin has been investigated by many authors. SCHÄFER (1872) found no effects in 0.2–0.4% arsenic acid; later, CITTENDEN and ALLEN (1886) found that the digestion was slightly accelerated in 0.2–0.5% arsenic acid and in 0.2–1% arsenious acid, while in 2–10% arsenious acid, it was repressed. In 1908, ASCHER found the repression of digestion in 0.125–0.25% atoxyl solution. PINCUSSOHN (1908) found that there was repression of digestion in the concentration of 1/500–1/3000 parts of 20% colloidal arsenic, but that in 1/60000 there was no effect. Further, SMORODINZEW (1924) showed that Na_2HAsO_5, K_5HAsO_4, and Na_2HAsO_4, in the concentration of higher than N/40, repress the digestion, while in N/80–N/10240 they have no effect.

Experiment IX.

(a) The order of addition. (Pepsin + Edestin) + Atoxyl.

TABLE IX.

No.	Solution.	Time of observation. (minutes)					
		5′	15′	30′	45′	50′	60′
		mm.	mm.	mm.	mm.	mm	mm
1	Control	12.4	17.7	25.6	35.2	—	44.2
2	1% Atoxyl	12.7	18.7	27.7	40.2	45.2	—
3	0.1% ,,	12.3	17.5	25.2	35.5	—	41.6
4	0.001% ,,	12.4	17.2	25.4	35.4	---	44.6

In 1% atoxyl the digestion was accelerated, but in a weaker concentration there was no effect. (Table IX and Fig. 5).
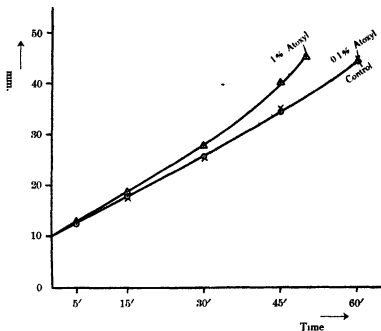
Fig. 5 The order of addition. (Pepsin + Edestin) + Atoxyl.

Experiment X.

(*b*) The order of addition. (Pepsin + Atoxyl) + Edestin.

TABLE X.

No.	Solution	Time of observation (minutes)				
		5′	15′	30′	45′	60′
		mm.	mm.	mm.	mm.	mm.
1	Control	12.4	16.7	25.5	34.3	44.4
2	1% Atoxyl	11.8	14.8	19.7	24.8	30.4
3	0.1% ,,	12.3	16.8	23.7	31.7	39.7
4	0.001% ,,	12.4	17.1	25.2	35.0	44.9

As may be seen from this result, when pepsin and atoxyl were first mixed, as well in 1% as in 0.1% atoxyl, the digestion was repressed. (Table X and Fig. 6).
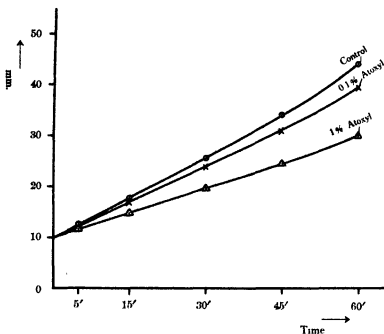
Fig. 6. The order of addition. (Pepsin+Atoxyl)+Edestin

Experiment XI.

(c) The order of addition. (Edestin + Atoxyl) + Pepsin.

TABLE XI.

No.	Solution.	Time of observation. (minutes).				
		5′	15′	30′	45′	60′
		mm	mm.	mm	mm	mm.
1	Control	12.2	17.1	25.4	34.1	44.3
2	1% Atoxyl	12.5	19.0	28.4	40.3	..
3	0.1% ,,	12.3	17.2	25.1	33.9	43.9

In the 1% solution of Atoxyl a remarkable acceleration occurred. (Table XI and Fig. 7).

In the case where the addition of edestin was the last, the diges-
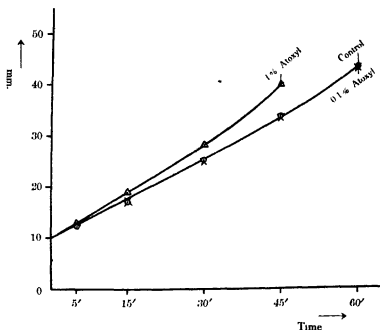
Fig 7. The order of addition. (Edestin+Atoxyl)+Pepsin.

tion was repressed, but when atoxyl and edestin were first mixed acceleration of the digestion was noticed. From these experiments it may be concluded that the repressing action of atoxyl on pepsin is not only eliminated by the presence of protein, but also that atoxyl can accelerate the action of pepsin according to circumstances.

Experiment XII.

In this experiment, atoxyl was used relatively to the acidic condition,* to determine whether a difference of these conditions affects the action of atoxyl on pepsin, as it is rather probable that the dissociation of atoxyl depends upon the reaction of the solution. The order of addition was the same as in Experiment X, but the pepsin

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* As atoxyl in a 1% solution is precipitated by the addition of hydrochloric acid, the poison was previously warmed and then mixed with pepsin which was acidified with hydrochloric acid.

solution was acidified with hydrochloric acid and the acidity of the
edestin solution itself was diminished to a corresponding degree.
Results are given in Table XII and Fig. 8.

TABLE XII.

No	Solution.	Time of observation. (minutes).				
		5′	15′	30′	45′	60′
		mm.	mm	mm.	mm.	mm.
1	Control	12.0	15.7	22.4	30.0	40.4
2	1% Atoxyl	12.2	15.6	22.8	31.0	40.8
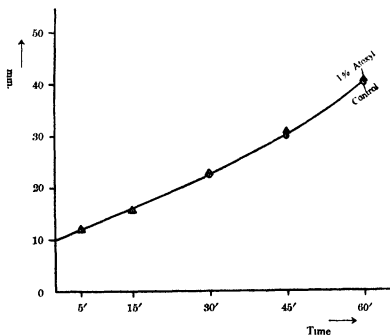
Fig. 8. The same order of addition as in Fig. 6. Pepsin acidified

The result from the mixture with 1% atoxyl was different from
that of the former experiment and there was no effect by the addition
of atoxyl. Further studies are necessary in any case to settle this
problem conclusively.

III SUMMARY.

1) In connection with the result of experiments which were carried out to determine the property of the proteolytic enzyme of *Drosera rotundifolia*, the effect of quinine-hydrochloride and atoxyl on pepsin was studied.

2) The effect of quinine-hydrochloride.

    a) The order of addition. (Pepsin + Edestin) + Quinine.

In 1% quinine-hydrochloride, the digestion was repressed while in 0.001-0.1% solution no effect was evident.

    b) The order of addition. (Pepsin + Quinine) + Edestin.

In 0.1%-1% solution, the result was almost the same as that of (*a*). But when edestin was added after the quinine had acted on the pepsin for a given time, even in its comparatively weak concentrations the digestion was clearly repressed.

    c) The order of addition. (Quinine + Edestin) + Pepsin.

0.1-1% quinine-hydrochloride solutions show no effect upon digestion.

    d) Quinine-hydrochloride acidified with hydrochloric acid was tried in the same order of addition as in (*b*). In this case the repression was weaker than in (*b*).

3) The effect of atoxyl.

    a) The order of addition. (Pepsin + Edestin) + Atoxyl.

In 0.1-1% atoxyl solution, the digestion was accelerated while in 0.001% solution no effect was evident.

    b) The order of addition. (Pepsin + Atoxyl) + Edestin.

In 0.1-1% atoxyl solution, the digestion was repressed while in 0.001% solution there was no effect.

    c) The order of addition. (Edestin + Atoxyl) + Pepsin.

The result was the same as in (*a*).

    d) When pepsin was acidified and the atoxyl and edestin were mixed in the same order as in (*b*), the digestion in the solution with 1% atoxyl was neither accelerated nor repressed.

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Mitosen im keimenden Embryo von *Sargassum* *Horneri* (Turn.) Ag.

Von

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(Mit Tafel XXV–XXVI).

Im vorigen Jahre habe ich eine Mitteilung über die Vorgänge der Reduktionskernteilung im Oogonium von *Sargassum Horneri* publiziert. In einem wichtigen Punkt ist mein Resultat ganz verschieden von dem eines frühren Forschers, Kunieda. Ich konnte nämlich mit Sicherheit bei der Reduktionsteilung 32 haploide Chromosomen zählen, während Kunieda nur etwa 16 Chromosomen bei derselben Teilung bemerkte.

Um die Karyokinese dieser Alge noch näher zu studieren, begab ich mich in diesem Frühling wieder nach Misaki, in die Biologische Station der Kaiserlichen Tokyo-Universität. Ich fixierte Antheridien und junge Embryonen von *Sargassum Horneri* reichlich mit verschiedenen Chromessigosmiumsäurelösung, aber es war sehr schwer, eine gute Kernfixierung zu erhalten. Da mir eine befriedigende Fixierung der Antheridien diesmal nicht gelang, bin ich hier leider nur imstande, einige Angaben über die somatische Kernteilung in den jungen Embryonen zu machen.

Als Material brauchte ich junge, zwei bis achtzellige Embryonen, die zwei oder drei Tage nach Oogoniumentleerung noch mit dichtem Schleim auf dem Rezeptakel der Mutterpflanze befestigt gefunden wurden. Noch weiter fortgeschrittene Stadien der Embryoentwicklung sind für die Untersuchung nicht geeignet, weil sich die Zellkerne immer mehr verkleinern.

Folgende Flüssigkeit fixierte die verschiedenen Phasen der Kernteilung verhältnismässig gut.

      Vorratslösung von Chromsäure (Seewasser 98 ccm, gesättigte
      Lösung von Chromsäure 2 ccm) ....50 ccm.
      Seewasser ....50 ccm.
      2% Osmiumsäure ....5 ccm.

Eisessigsäure ....2.5 ccm.

Die Färbung der Paraffinschnitte erfolgte ausschliesslich mit HEI-
DENHAINS Eisenalaunhämatoxylin.

Über die Frage, wo die erste Teilung des befruchteten Kerns in
der Eizelle von *Sargassum* beginnt, gibt es zwei verschiedene Meinungen.
TAHARA und SHIMOTOMAI (1926) bemerkten bei *Sargassum toritile*,
dass die erste Teilung des befruchteten Eikerns immer in der Peri-
pherie der Eizelle erfolgt, aber KUNIEDA (1928) hat ein anderes
Resultat mitgeteilt: bei *Sargassum Horneri* vollziehe sich die Befruch-
tung und die ihr folgende erste Kernteilung nicht in der Peripherie
der Eizelle, sondern im Zentrum. Da meine Beobachtungen noch
sehr spärlich sind, kann ich zunächst zu diesem wichtigen Problem
nichts sagen.

Der Ruhekern ist meist kugel- oder eiförmig oder ellipsoidisch.
Er enthält gewöhnlich ein, zuweilen zwei Nukleolen und viele kleine
Chromatinkörner, die mit ausserordentlich zarten Faden rosenkranzartig
verbunden und in der Kernhöhle gleichmässig verteilt sind. Das
Plasma in der Umgebung des Kerns ist etwas dicht. Die Zellen teilen
sich ohne Zunahme ihres Volumens so schnell, dass die Telophasekerne
gewöhnlich nach kurzer Ruhe ins Prophasestadium der nächsten Teilung
übergehen.

Im frühesten Stadium der Prophase, als Zeichen des Beginns der
Kernteilung, tritt eine merkliche Veränderung, d. h. eine Strahlung
im Plasma, auf einer Seite der Kernmembran auf. Wie in FIG. 1
deutlich gezeichnet ist, kann man zwei kleine, etwas gekrümmte,
stäbchenförmige Körnchen in der Mitte dieser Strahlung, in der Nähe
der Kernmembran erkennen. Bei genauer Betrachtung lässt sich auch
wahrnehmen, dass diese Stäbchen in einen hellen Hof gebettet sind.
Bei *Stypocaulon* bemerkte SWINGLE (1897), dass ein solches Körnchen
im Beginn der Karyokinese in zwei geteilt wird. Bei *Sargassum*
konnte ich aber diese Erscheinung nicht beobachten. Wie unten noch
erklärt wird, gibt es schon in der Prophase, der vorhergehenden
Teilung je zwei Körnchen im Zentrum der Strahlung. Die Termino-
logie des Zentralapparats ist in der Literatur etwas verwirrt. Der
Einfachheit halber möchte ich hier dieses Paar von Körnchen mit
hellem Hof als Zentrosom bezeichnen. Die zarten Kernfäden sind
geneigt, nach der Seite mit der Strahlung zu verlaufen (FIG. 1).

Im nächsten Stadium erscheinen zwei Strahlungen, die vielleicht durch Zweiteilung der vorher vorhandenen entstanden sind (Fig. 2). Merkwürdigerweise hat jede Strahlung im ihrem Zentrum zwei Körnchen. STRASBURGER (1897) hat das gleiche Verhältnis bei der Untersuchung von *Fucus* gefunden — besonders deutlich in seiner Fig. 51 —, während SWINGLE in demselben Stadium bei *Stypocaulon* an jedem Pol nur ein nicht geteiltes, hantelförmiges Körnchen bemerkte.

Mit dem Fortschreiten des Stadiums zerschneiden sich die Kernfäden an verschiedenen Stellen und gestalten sich allmählich zu Chromosomen, die in der Kernhöhle regellos zerstreut sind (Fig. 3 und 4). Wie bei der Prophase der Reduktionsteilung dieser Pflanze, trennen sich die beiden Strahlungen entlang der Kernwand, aber niemals ganz bis zur gegenseitigen Stelle des Kerns. Die Zentrosomen entfernen sich wenig von der Kernwand, und die Fasern wachsen nach dem Kern hin, so dass die Kernwand an diesen Punkten etwas eingedrückt ist und schliesslich dort aufgelöst wird (Fig. 3, 4 und 5).

Zur Zeit des Eintritts der Spindelfäden in die Kernhöhle sammeln sich die bisher im ganzen Raum des Kerns zerstreuten Chromosomen in der Gegend, wo die Enden beider eintretender Spindelfaserbüschel zusammentreffen (Fig. 5). Wenn sich eine intranukleäre Spindel auf einer Seite des Kerns bildet, so ordnen sich die Chromosomen auf der Kernplatte an. Der Nukleolus verschwindet dann allmählich, aber die Kernmembran bleibt noch intakt (Fig. 6 und 7). Gewöhnlich löst sich die Kernmembran bei vollständiger Metaphase gänzlich auf, und kleine, stäbchenförmige Chromosomen verteilen sich regelmässig auf einer Äquatorialplatte (Fig. 8).

In der Polansicht solcher Kernplatte konnte ich feststellen, dass die Zahl der Chromosomen mit grosser Wahrscheinlichkeit 64 beträgt (Fig. 9). Sie ist somit doppelt so gross wie im Kerne des Oogoniums, der im vorigen Jahre von mir untersucht wurde.

Mit dem Beginn des Anaphasestadiums erfolgt die Längsspaltung aller Chromosomen. Darauf rücken die beiden Längshälften regelmässig in die entgegengesetzte Richtung auseinander (Fig. 10). Der Zwischenraum zwischen beiden Massen der Tochterchromosomen dehnt sich allmählich aus und wird plasmaarm (Fig. 11 und 14).

Wie Fig. 13 zeigt, erscheint zuweilen ein eigentümliches Anaphasestadium, wo die Kernwand noch intakt bleibt, während die Tochter-

chromosomen schon wandern.

Das Telophasestadium scheint sich langsam zu entwickeln, da man die verschiedenen Stufen der Ausbildung der Tochterkerne in einem Präparat gut beobachten kann. Die Tochterchromosomen, die die Pole erreicht haben, ziehen sich zunächst in eine kleine Masse zusammen. Die Vakuolisierung der Chromosomen tritt polwärts nach und nach, zugleich mit Neubildung der Kernmembran (Fig. 14). Das Chromatingerüst der Tochterkernanlage ist zuerst an der Polseite der Kernmembran gedrückt, dehnt sich dann aber in der Kernhöhle aus, und gewöhnlich kommen zwei, drei oder noch mehr Nukeolen zum Vorschein.

Bei der Untersuchung der gleichen Stadien von *Fucus* hat STRAS-BURGER etwas sehr Interessantes gefunden. Er bemerkte nämlich, dass das zugespitzte Ende der Tochterkernanlage immer von dem Zentrosom eingenommen wird. Bei *Sargassum Horneri* ist aber das Verhältnis ganz anders. Denn die Tochterkernanlage dieser Pflanze orientiert sich, wie Fig. 14–16 klar zeigen, von dem Zentrosom etwas entfernt, und die dem Zentrosom zugekehrte Seite der Membran ist nicht zugespitzt, sondern etwas abgeplattet. Aber wenn die Ausbildung des Tochterkerns ungefähr vollendet ist, nähert sich das Zentrosom der Kernmembran (Fig. 17).

Nach voller Ausbildung der Tochterkerne wird die Zellwand zwischen den beiden Kernen zentripetal gebildet.

ZUSAMMENFASSUNG.

1. In der Prophase der somatischen Kernteilung im Embryo von *Sargassum Horneri* treten zuerst eine, dann zwei Strahlungen auf.

2. Während aller Teilungsstadien besitzt die Strahlung in der Mitte zwei kleine, etwas gekrümmte Stäbchen.

3. Wenn sich die Spindel auf einer Seite der Kernhöhle bildet, ordnen sich die Chromosomen auf der äquatorialen Ebene.

4. Die Kernmembran löst sich gewöhnlich in der Metaphase auf, bleibt aber zuweilen noch bis zur Anaphase intakt.

5. Diese Pflanze zeigt 64 Diploidchromosomen.

6. In der Anaphase wandern die Tochterchromosomen nicht bis zum Zentrosom. Also kommt die Tochterkernanlage in der Telophase

mit dem Zentrosom nicht in Berührung.

Zum Schluss möchte ich Herrn Prof. Dr. M. Tahara für seine Anregung und seine vielseitigen Ratschläge meinen herzlichen Dank aussprechen. Ebensoviel Dank schulde ich auch Herrn Prof. Dr. N. Yatsu, Direktor der Biologischen Station zu Misaki, für seine freundliche Unterstützung.

den 27. Oktober 1930.

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ERKLÄRUNG DER TAFELN

Sämtliche Abbildungen wurden mit Hilfe eines Abbéschen Zeichenapparats gezeichnet, unter Benutzung des Zeisschen Achromat-Objektivs $\frac{1}{12}$ und des Zeisschen Okulars ×20. Vergrösserung ca. 1900.

TAFEL XXV.

Fig. 1. Frühere Prophase, wo eine Strahlung erscheint.

Fig. 2. Späteres Stadium, wo die zwei Strahlungen zu sehen sind

Fig. 3–4. Noch spätere Stadien. Die Chromosomen sind in der Kernhöhle zerstreut. In Fig. 4 beginnen die Spindelfasern in den Kern einzudringen.

Fig. 5–6. Spätere Telophasen.

Fig. 7. Dasselbe Stadium in Polansicht.

Fig. 8. Metaphase in Seitenansicht.

Fig. 9. Dasselbe Stadium in Polansicht. wobei 64 Chromosomen zu sehen sind.

TAFEL XXVI
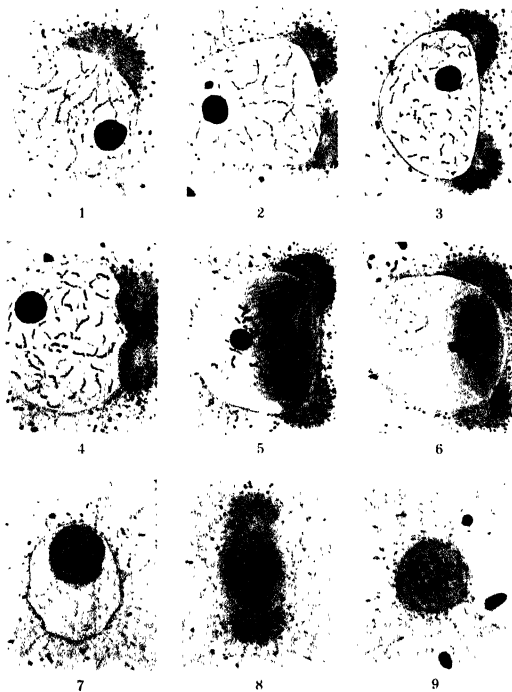
S. OKABE: Mitosen im Embryo von *Sargassum*.

Effect of Light on Porphyrin, from the Integument of the Earthworm, Allolobophora foetida (Sav)

By

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(With 3 text figures).

INTRODUCTION

According to ZIELINSKA (1913) and FISCHER and SCHAUMANN (1923), the pigment of the earthworm *Eisenia foetida* differs from haematoporphyrin discovered by MAC MUNN (1886) in the body wall of *Lumbricus terrestris* both spectroscopically and chemically The present writer (1928) also studied this pigment of *Allolobophora foetida* by the Essigathermethode of FISCHER and SCHAUMANN and found that the absorption bands of this pigment shifted further towards the red than those of haematoporphyrin

HAUSMANN (1916) extracted this pigment from *Eisenia foetida* with hydrochloric acid alcohol and found that the fluorescent solution so obtained produces photodynamical sensitivity to erythrocytes.

In my subsequent observation on this pigment, it was noted that the characteristic positions of the absorption bands altered after exposure to sunlight, and that its colour faded in this solution

Similar phenomena to these just mentioned were noted by several workers who employed different pigments for instance SCHUMM (1914), GOTO (1923), and KAJDI (1925) on haematoporphyrin SCHUMM (1916) and SQUIRES (1927) on uroporphyrin

In 1923 FISCHER and KÖGL noted in the ooporphyrin ester from the egg-shell and further noticed that the colour of the solution became green and the characteristic bands disappeared producing at the same time, a new additional band in the red region at 676 0 661 2 after irradiation for one hour with an arc lamp

So far as I am aware, the spectoral changes of worm porphyrin in relation to the exposure to light have not been reported and I have therefore atempted to study this problem

EXPERIMENTAL METHOD.

2 kilograms of the earthworm, *Allolobophora foetida*, were collected in June, 1929. Since the " Essigäthermethode " used in my previous work, yields too smaller amount of the materials, the other method which is described below was substituted. The entire procedure was carried on in a dark room in order to prevent the effect of exposure to sunlight.

The alimentary tract was cleaned and the coelomic fluid, which was secreted from the dorsal pores of the worm, was removed by the method employed in my previous work (1928). The worms thus cleaned were finely chopped with a meat grinder and washed with running water for 24 hours in order to remove blood pigment.

For extracting the pigment, the tissue was first extracted with 2 liters of 20% hydrochloric acid alcohol, and was then allowed to stand for several days with occational shaking. In the extractives thus obtained two characteristic absorption bands could be seen by means of the spectroscope.

The residue was strained first through cheese cloth, then centrifugalized, and the remaining sediment was finally removed from the filtrate by filter paper. The filtrate was neutralized by a concentrated potassium hydroxide solution and was then mixed with ether into which the porphyrin passed. The ethereal solution of the porphyrin was washed several times with water in order to remove the major portion of alcohol and inorganic salts. The glacial acetic acid was added and it was shaken vigorously. The porphyrin, in the glacial acetic acid, was again passed into the ether by diluting the acidity with water. The ethereal solution of porphyrin thus separated, was redissolved in 20% hydrochloric acid instead of glacial acetic acid and was extracted by ether in neutralizing acidity with potassium hydroxide solution. The ethereal solution so obtained was finally washed with a large amount of distilled water till the solution was free from acid and alkalies. On evaporating the ether, the worm-porphyrin, exhibiting a dark brown colour, which was used for the present work was obtained in a powdered form. The yield of worm-porphyrin, was about 1.5 grams.

The worm-porphyrin was studied both spectrometrically and spec-

trophotometrically. For the spectrometric study, ADAM HILGER's wave length spectrometer (Constant Deviation Type) was employed for determining the axis of the absorption spectra. As the light source, a carbon arc lamp (4–5 ampares) was employed, and, as the container of the worm-porphyrin solution, BALY's absorption tube was used. To measure the quantitative changes of the absorption spectra, KÖNIG-MARTENS's spectrophotometer (model II) by SCHMIDT and HAENSCH was employed. These instruments were carefully adjusted before use; the scale of the instrument was calibrated by observing the known spectra of various metals for which the values of the wave length were indirectly calculated for given scale readings (see appendix, Table 2). The opening of the colimater slit was fixed to 0.1 mm. The light source for the instrument was the paralleled light of a concentrated filament from a 250 watt lamp.

EXPERIMANTAL RESULTS

The worm-porphyrin was dissolved in the following solvents; 20% hydrochloric acid alcohol, 25% hydrochloric acid, 6 and 3% acetic acid alcohol, ether, ethylalcohol, amylalcohol, 20% ammoniacal alcohol, and 5% potassium hydroxide alcohol. Most of these experiments were carried out with the spectrometer but some with the spectrophotometer. The solutions were exposed to sunlight with the exception of the pigment in acetic acid alcohol which was exposed to the irradiation of an electric lamp. For the exposure to sunlight, the solutions were put in glass vessels 2.6 cm. in diameter and 1 cm. in height, and then covered with a quartz plate.

A. Spectrometric changes of worm-porphyrin.

The worm-porphyrin in 20% hydrochloric acid alcohol showed two well-defined bands at 604 and at 559. The latter band is much more intense than the former.

By the exposure to sunlight, the original wine-red colour of the solution turned to yellow and gradually faded away. However this fading of the colour produced little or no change in the positions of the bands, though no bands could be seen when the colour had entirely disappeared (See Table 1.). The solution made with 25%

hydrochloric acid also showed two bands at corresponding positions
with the former. By the irradiation to light for 5 minutes, the band
which was observed at 603 in the unexposed solution, suddenly changed
its position and also divided into two axes at 612 and 596, yet the
band at 559 remained unchanged. After exposing for 15 minutes,
only the band at 612 could be seen, while the band at 596 became
invisible. Further exposure to the irradiated light produced a complete
disappearance of all bands was the case in the 20% hydrochloric acid
alcoholic solution (see Table 2).

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TABLE 1.

Absorption bands in 20% HCl alcohol		Time of exposure
604	559	Before exposure
604	559	5′
604	559	15′
604	559	45′
—	559	2 hrs. 45′
—	—	6 „

TABLE 2.

25% hydrochloric acid		Time of exposure
603	558	Unexposured
612 596	558	5′
612	558	15′
612	558	45′
612	558	1 hr. 45′
	559	2 hrs. 45′

</div>

In general the absorption spectra of either the exposed or un-
exposed solutions made with acetic acid alcohol, ether, ethylalcohol,
and amylalcohol resemble each other, although a slight variation in
the position of the axes may be found according to the solvents used.
Tables 3–6 give the spectral changes in various solvents.

TABLE 3.

				Absorption bands in 6% acetic acid alcohol					Time of exposure
		631	60(6)	583			541	504	Before exposure
66(9)	64(8)	630	60(6)	582		555	540	503	5′
67(0)	648	629	60(6)	581	555	555	540	503	10′
67(0)	648	626	—	581	555	555	539	502	30′
67(0)	648	625	—	58(0)	555	555	537	50(2)	2 hrs.

TABLE 4.

				Absorption bands in ether					Time of exposure
		634	606	584	576		537	503	Before exposure
670		634	606	—	576	553	537	503	5′
670		634	606	—	576	553	537	503	15′
670	648	633	606	—	576	553	537	503	30′
669	649	633	—	--	575	554	53(4)	502	1 hr. 30′

TABLE 5.

				Absorption bands in ethylalcohol					Time of exposure
		630	60(3)	583	575		537	504	Before exposure
670		630	60(3)	583	575		537	503	5′
670		630	60(3)	583	575	55(7)	537	503	15′
670	649	630	60(3)	583	575	556	537	503	1 hr 30′
669	647	629	—	582	575	556	536	502	2 hrs
669	646	625		581	—	556	535	502	4 ,,
668	646	623		582	—	556	534	502	5 ,,

TABLE 6.

				Absorption bands in amylalcohol					Time of exposure
		631	60(4)	584	576		539	504	Before exposure
671	649	631	60(4)	58(4)	575		539	504	5′
671	649	631	60(4)	—	576		538	504	15′
671	649	631	60(4)	—	575		538	504	1 hr
671	648	630	60(4)	—	575	55(7)	538	503	2 hrs
671	649	631	.	—	575	55(6)	538	503	3 ,,
671	649	630	—	—	575	557	538	502	5 ,,
671	649	631	—	. .	—	558	537	501	6 ,,

The figures in brackets are uncertain.

From the above tables, the banded spectra are obtained in the unexposed worm-porphyrin; I at 634-630, II at 584-583 or 576-575 (the latter is not evident in acetic acid alcohol), III at 541-537 and, in addition, there is a very faint band at 606-603.

After exposure to sunlight, two new additional bands are developed at the red region near the infra red. The axis of the first band is at 671-669, and that of the second, at 649-648. The first band is

more intense than the second.

Another band also appears at 557-553 in the blue region, but it is not found in the case of haematoporphyrin. The axes of the absorption bands which are visible in the unexposed solution change their positions towards the violet and, at the same time, tend to decrease an absorption intensity of each band. After exposure for several hours, both bands become invisible.

In both the ammoniacal and potassium hydroxide alcoholic solutions (see Tables 7 and 8), the general behavior of the spectral changes is almost similar to that of the cases mentioned above. These two kinds of solutions possess the four banded spectra if unexposed, as was observed with the solutions made with the neutral solvents. However after exposure, the two new bands develop at 669-668 and at 649. In ammoniacal alcoholic solution, the faint band appears at 603 instead of at 649, as will be seen in Table 7.

TABLE 7.

Absorption bands in 20% NH₄OH alcohol							Time of exposure
	630		585	574	540	504	Before exposure
668	630		584	574	540	504	5′
668	630	603	584	574	540	504	15′
668	630	603	—	574	539	504	30′
668	630	604	-	573	539	503	1 hr. 30′
668	630	605	-	572	539	503	3 hrs.
669	629	606	-	570	539	502	5 ,,

TABLE 8.

Absorption bands in 5% KOH alcohol						Time of exposure
		629	574	537	503	Before exposure
669	649	628	573	536	502	5′
669	649	628	573	536	502	15′
670	649	629	57(3)	536	50(1)	45′
670	648	628	—	536	—	1 hr. 45′

The figures in brackets are uncertain.

B. Spectrophotometric changes of worm-porphyrin.

The spectral changes of the worm-porphyrin were also observed quantitatively in some of the following solvents; hydrochloric acid alcohol, ammonical alcohol, and acetic acid alcohol.

Fig. 1. give the spectrophotometric changes of worm-porphyrin in hydrochloric acid alcohol, affected by the irradiation of sunlight. Table 9 shows the spectrophotometric axis of the band. The axes of the bands are at 603 and 559 before exposure. The irradiation of light produces little or no change in the position of each band. Spectrophotometrically, a great difference between the exposed and unexposed worm-porphyrin lies in the fact that in the former the absorption intensity of each band tends to decrease gradually, and after 20 hours there are no bands remaining visible.
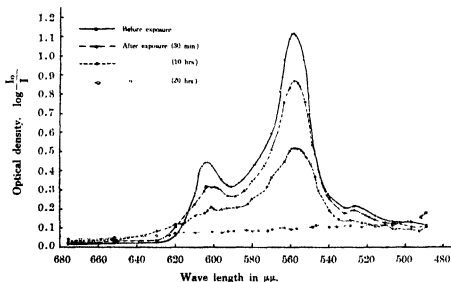
Fig. 1. Spectrophotometric curves of worm-porphyrin in 20% hydrochloric acid alcohol

In ammoniacal alcohol, four banded spectra were obtained, as is shown in Fig. 2 and Table 10. By exposing this solution to light, a new additional band at 669 gradually developed and its absorption intensity also gradually increased, while in the third and fourth bands the absorption intensity gradually diminished as the slight positional

TABLE 9. Spectrophotometric axes of the bands in
hydrochloric acid alcoholic solution.

Designation of band Time of exposure	I	III
Before exposure	603	559
After exposure (30 min)	603	558
After exposure (10 hrs)	60(1)	558
After exposure (20 hrs)	—	—

The figure in bracket is uncertain
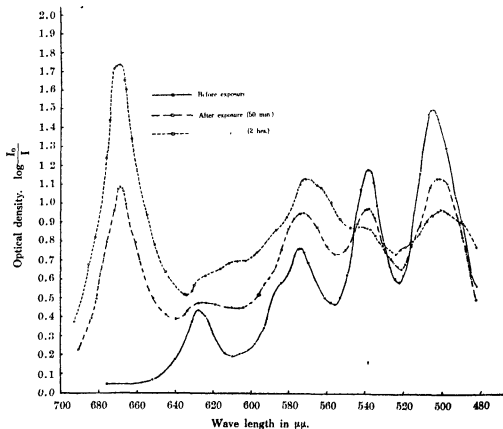
Fig 2. Spectrophotometric curves of worm-porphyrin in 20% ammoniacal alcoholic solution.

changes took place. The moving of each band agreed with that of
the spectrometric measurement (see Table 10 and Table 8.).

TABLE 10. Spectrophotometric axes of the bands in
ammoniacal alcoholic solution.

Designation of band Time of exposure	New band	I	New band	II	III	IV
Before exposure		628	—	574	538	504
After exposure (50 min)	669	62(6)	—	573	539	502
After exposure (2 hrs)	669	—	60(6)	571	uncertain	501

The figures in brackets are uncertain

I have observed a peculiar spectral change of the solution in acetic
acid alcohol. This change occurred after exposing the solution to the
irradiation of electric lamp light for four hours. As will be seen in
Fig. 3 and Table 11, the four banded spectra began to show two
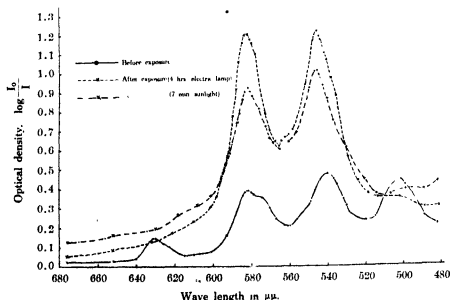
Fig. 3. Spectrophotometric curves of worm-porphyrin in 3% acetic acid alcohol

high summits at 581 and 545, and, at the same time, the absorption
intensity increased much more strongly than that shown by the un-
exposed solution. However, when this same solution was again exposed
to sunlight for seven minutes, the intensity of both absorption bands
showed a slight diminution.

TABLE 11.   Spectrophotometric axes of the bands in
acetic acid alcoholic solution.

Designation of band	I	II	III	IV
Time of exposure				
Before exposure	630	581	540	503
After exposure (4 hrs electric lamp)		581	545	
After exposure (7 min sun light)	—	581	545	—

From the fact that the worm-porphyrin possesses axes of the
bands at different wave lengths and that the spectrophotometric curves
are found to cross with each other, it seems evident that the worm-
porphyrin changes its own property by means of the irradiated light.

Furthermore, comparing the newly developed bands of worm-
porphyrin at 670–669 and 649–618, with those of haematoporphyrin
at 661–660 and 646–641 (KAJDI, 1925), it was found that the two
bands in the former shifted more toward the red than in the latter,
on the one hand, while the new band at the blue region given by
worm-porphyrin, is not given by haematoporphyrin, thus indicating
that these two pigments do not appear to be identical.

GOTO (1923) demonstrated a spectral change of haematoporphyrin
in acetic acid alcohol which resembles closely that of the worm-por-
phyrin as the following table shows :

Haemato-porphyrin	571	563	538	1 hr.	Irradiation of metal flame light.
,,		561	538	2 hrs.	,, ,, ,, ,, ,,
Worm-porphyrin		581	545	4 hrs.	,, ,, electric lamp.

We thus find that the axes of the bands given by the worm-
porphyrin are not strictly identical with those given by |

porphyrin, since in the former the worm-porphyrin are shifted more to the red than in the latter.

I may add that the spectra of the unexposed worm-porphyrin also shifted more to the red compared with those of haematoporphyrin.

On the other hand, FISCHER and SCHAUMANN (1923) determined the axes of the worm-porphyrin in ether as:

633.2–635.6   589.5–568.4   548.3–529.1   515.5–495.2

These positions almost agree with the data given in Table 4, though considerable deviations can be found in the band placed nearer the red region.

When the data given in my previous work (1928) on the spectra of the worm-porphyrin dissolved in hydrochloric acid alcohol and ammoniacal alcohol, are compared with the similar data given in the present work, some discrepancies in the result are noted. This difference may be considered to be due to irradiation during the operation made in the former investigation, while in the present study such exposure to light was avoided by preparing the materials in a dark room.

SUMMARY.

The results obtained from the above experiment show that the absorption spectra of the worm-porphyrin are altered by the irradiation to light as is the case with other porphyrins; e. g. haematoporphyrin, uroporphyrin, and ooporphyrin.

The axes of the bands in worm-porphyrin, when exposed to light, are shifted to the red and also the axes of the new additional bands thus produced are found in the region, contrasted with those of the corresponding bands of haematoporphyrin. Such spectral changes are found to occur within a very short period of exposure.

The worm-porphyrin extracted from the integument of the earthworm *Allolobophora foetida* differs spectroscopically from the ordinary haematoporphyrin.

The results of this study support strongly the view held by HAUSMANN (1916) that this pigment possesses photodynamic sensitisation against the erythrocytes.

The present results show also that the pigment changes its own property as the results of beeing exposed to irradiated light.

In conclusion, I desire to acknowledge my sincere thanks to Prof. S. HATAI for his valuable suggestion and criticism throughout the course of this work, and my thanks are also due to Prof. J. ÔKUBO of the Physical Institute, for his help in the technique to spectroscopy.

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APPENDIX.

Spectrophotometric values from which the curves of Fig. 1, 2 and 3 were constructed and the values in Table 1 are shown as the optical density, $\log \dfrac{I_0}{I}$.

TABLE 1.

Wave length in mμ	Hydrochloric acid alcoholic solution				Acetic acid alcoholic solution			Ammoniacal alcoholic solution		
	Before exposure	Exposed for 30 min	Exposed for 10 hours	Exposed for 20 hours	Before exposure	Exposed for 4 hours (Electric lamp)	Exposed for 7 min (Sunlight)	Before exposure	Exposed for 50 min	Exposed for 2 hours
693	—	—	—	—	—	—	—	—	—	0.371
691	—	—	—	—	—	—	—	—	0.229	—
688	—	—	—	—	—	—	—	—	—	0.678
684	—	—	—	—	—	—	—	—	0 391	—
682	—	—	—	—	—	—	—	—	—	0.815
681	—	—	—	—	—	—	—	—	0.496	—
676	0.018	0.021	0.031	0.044	0.022	0.054	0.128	0.046	0.799	1.235
673	—	—	—	—	—	—	—	—	—	1.434
672	—	—	—	—	—	—	—	—	0 960	1.711
671	—	—	—	—	—	—	—	—	—	1 718
670	—	—	—	—	—	—	—	—	1.076	—
669	—	—	—	—	—	—	—	—	1.087	1.731
668	—	—	—	—	—	—	—	—	1.078	—
667	—	—	—	—	—	—	—	—	1.061	1.640
666	—	—	—	—	—	—	—	—	0.999	—
665	—	—	—	—	—	—	—	—	—	1.600
663	—	—	—	—	—	—	—	—	—	1.338
661	—	—	—	—	—	—	—	—	0.793	—
655	—	—	—	—	—	—	—	—	—	0.937
652	0.026	0.036	0.040	0.052	0.030	0.091	0.164	0 068	0.198	—
651	—	—	—	—	—	—	—	—	—	0.782
645	—	—	—	—	—	—	—	—	0.409	0.639
640	—	—	—	—	0.042	—	—	0.175	0.391	—
635	—	—	—	—	—	—	—	—	0.404	—
634	—	—	—	—	0.115	—	—	—	0.430	0.518
632	—	—	—	—	0.136	0.132	—	—	—	0.510
631	—	—	—	—	0.141	—	—	0.374	—	—
630	0.027	0.036	0.084	0.066	0.143	—	0.199	0.402	—	0.535
628	—	—	—	—	0.154	—	—	0.432	0.470	0.561
626	—	—	—	—	0.121	—	—	0.420	0.474	—
624	—	—	—	—	—	—	—	0.402	0.471	—
623	—	—	—	0.068	0.102	—	—	—	—	0 610
621	—	—	—	—	—	—	—	—	—	—
620	0.062	0.110	0.115	0.078	—	0.178	—	—	0.467	—
619	—	—	—	—	—	—	—	—	—	—
618	—	—	—	—	—	—	0.268	—	—	—
616	—	—	0.124	—	—	—	—	—	—	—
615	—	—	—	—	0.059	—	—	—	—	0.650
614	—	0.180	—	—	—	—	—	0.207	—	—

Wave length in mμ	Hydrochloric acid alcoholic solution				Acetic acid alcoholic solution			Ammoniacal alcoholic solution		
	Before exposure	Exposed for 20 min.	Exposed for 10 hours	Exposed for 20 hours	Before exposure	Exposed for 4 hours (Electric lamp)	Exposed for 7 min. (Sunlight)	Before exposure	Exposed for 50 min.	Exposed for 2 hours
610	0.281	0.224	0.169	0.078	0.066	0.232	—	0.189	0.446	0.680
608	0.388	—	—	—	—	—	0.314	—	—	—
607	—	—	—	—	0.068	—	—	—	—	—
606	0.423	—	—	—	—	—	—	0.202	0.440	0.690
605	—	0.295	0 178	—	—	—	—	—	—	—
604	0.432	—	—	—	—	—	—	—	0.447	—
603	0.433	0.318	0.194	—	—	—	—	0.210	—	—
601	0.426	0.316	0.200	0.080	0.077	0.304	0.362	—	—	0.689
600	—	—	0.198	—	—	—	—	—	—	—
599	—	0.315	—	—	—	—	—	—	—	—
598	—	0.310	—	—	—	0.360	—	0.257	—	—
596	0.351	—	0.199	0.077	—	—	0.436	—	0.509	0.729
593	0.329	0.273	0.204	0.083	0.160	—	—	0.341	—	—
592	—	—	—	—	—	0.568	—	—	—	—
591	0.318	—	—	—	—	—	0.587	—	—	—
590	—	—	—	0.084	—	—	—	—	—	—
589	—	0.275	—	—	—	0.777	—	—	—	—
588	—.	—	0.204	—	—	—	—	—	—	0.855
587	0.334	—	—	—	—	0.918	0.741	—	—	—
586	—	—	—	—	—	1.068	0.793	—	—	—
584	—	0.293	0.226	0.087	0.367	1.161	0.865	0.573	0.750	—
583	—	—	—	—	0.386	1.206	0.903	—	—	—
581	—	—	—	—	0.390	1.207	0.926	—	—	0.990
580	—	0.343	0.251	0.083	0.385	1.180	0.906	—	0.855	—
579	0.423	—	—	—	0.374	1.074	0.883	—	—	—
577	—	—	0.280	0.084	—	1.003	—	0.717	0.910	—
575	—	—	—	—	—	—	0.814	0.752	—	—
574	—	0.438	—	—	0.353	—	—	0.756	0.936	1.093
573	—	—	—	—	—	—	—	0.756	—	1.113
572	—	—	0.311	0.084	—	0.791	0.723	0.736	0.940	—
571	—	—	—	—	—	—	—	—	—	1.119
570	0.592	0.507	0.361	0.089	—	0.702	0.663	0.681	0.927	—
568	—	—	—	—	—	—	0.647	—	—	1.112
567	—	—	—	—	0.243	0.649	—	—	—	—
565	0.809	—	—	—	—	0.622	0.604	—	0.968	1.090
564	—	—	—	—	—	0.646	—	—	—	—
563	0.976	0.753	0.474	0.100	0.215	0.657	—	—	—	1.078
562	—	—	—	—	—	0.670	—	—	—	—
561	—	—	—	—	0.210	—	—	0.499	—	—
560	1.082	0.832	0.500	0.090	0.212	0.684	0.648	—	0.764	—
559	1.124	—	0.511	—	0.235	—	—	—	0.763	—
558	1.113	0.869	0.517	—	—	0.710	—	0.472	—	0.999
557	1.077	—	—	0.098	—	—	—	—	—	—
556	—	0.859	0.515	—	0.246	—	0.696	0.459	0.723	—
555	—	0.840	—	—	—	—	—	—	—	—
554	—	—	0.503	—	—	—	—	—	—	—
553	0.978	—	0.487	0.098	—	—	—	0.497	0.723	0.905
552	—	0.756	0.467	—	—	—	—	—	—	—

Wave length in μμ	Hydrochloric acid alcoholic solution				Acetic acid alcoholic solution			Ammoniacal alcoholic solution		
	Before exposure	Exposed for 30 min.	Exposed for 10 hours	Exposed for 20 hours	Before exposure	Exposed for 4 hours (Electric lamp)	Exposed for 7 min. (Sunlight)	Before exposure	Exposed for 50 min	Exposed for 2 hours
551	—	—	—	—	0.945	—	—	—	—	—
550	0.698	—	—	—	—	—	—	—	0.743	—
549	—	—	0.436	0.105	0.332	—	0.925	0.617	—	—
548	—	—	—	—	—	—	—	—	—	0.859
547	0.484	0.531	0.370	0.116	—	1.174	—	—	—	—
546	—	—	0.291	—	—	1.214	1.013	0.826	—	—
545	—	0.403	—	—	—	1.227	1.019	—	—	—
544	—	—	—	—	0.447	1.198	—	—	0.885	0.862
543	—	—	—	—	—	1.176	0.964	—	—	—
542	—	—	—	—	—	—	—	1.061	—	—
541	0.276	—	—	—	—	1.107	0.939	—	—	—
540	—	0.259	0.192	0.105	0.480	1.072	—	—	0.956	0.861
539	—	—	—	—	0.477	—	0.872	—	0.959	—
538	0.274	—	—	—	—	0.879	—	1.168	0.962	0.852
537	—	—	—	—	0.455	—	—	—	—	—
536	—	—	—	—	—	—	0.756	1.152	0.937	—
535	—	—	0.141	0.106	0.426	0.786	—	1.098	0.916	0.820
534	—	—	—	—	—	—	—	0.975	—	—
532	0.205	0.179	—	—	0.350	—	—	—	—	—
531	—	—	—	—	—	—	—	—	—	0.774
530	—	—	—	—	0.317	—	0.618	0.781	0.750	—
529	—	—	0.141	0.111	0.280	0.569	—	—	—	—
527	0.213	0.193	—	—	—	—	—	0.652	0.669	0.749
524	—	—	—	—	—	0.441	—	0.596	—	0.721
523	—	—	0.128	0.109	—	—	—	—	0.654	—
522	—	—	—	—	—	—	0.450	0.576	—	0.748
521	—	0.165	—	—	—	—	—	—	0.640	—
520	—	—	—	—	0.240	—	—	0.610	—	—
519	—	0.159	0.119	0.120	—	0.372	—	—	0.670	0.758
516	0.164	0.142	0.114	0.118	0.251	—	0.373	0.784	0.763	0.783
514	—	—	—	—	—	0.355	—	—	—	—
512	—	—	—	—	—	—	—	—	—	0.829
511	—	0.130	0.117	0.117	0.357	—	—	1.145	0.868	—
508	—	—	—	—	—	—	—	—	—	0.895
507	0.135	0.127	0.097	0.116	0.408	—	0.376	1.456	—	—
506	—	—	—	—	—	—	—	1.476	1.072	0.922
505	—	—	—	—	—	—	—	1.481	—	—
504	—	—	—	—	0.438	—	—	—	—	—
503	—	—	—	—	0.433	—	—	—	—	—
502	—	—	—	—	0.448	0.352	—	1.450	1.114	0.941
501	—	—	—	—	—	—	—	1.430	1.113	0.957
500	—	—	—	—	0.427	—	—	—	1.100	0.950
498	—	—	—	0.137	0.370	0.337	0.392	—	—	—
497	0.139	0.109	0.096	—	—	—	—	1.280	1.091	0.930
493	—	—	0.084	0.158	0.340	—	—	1.018	—	—
489	0.112	0.115	—	—	0.256	0.300	0.393	0.795	0.805	0.884
485	—	—	—	—	—	—	—	0.624	—	—
482	—	0.105	0.104	0.179	0.220	0.308	0.437	0.551	0.482	0.752

TABLE 2.

Metal	Wave length in μμ	Scale reading of spectrophotometer
Li	670.8	518.2
Si	640.1 *	506.1
Fe	613.7	491.9
Li	610.4	490.1
Na	589.3	478.0
Hg	579.1	471.2
Cu	578.2	470.5
Hg	577.0	470.2
Cu	570.0	465.1
Fe	561.6	460.5
Sn	556.4	455.5
Sr	548.1	449.0
Hg	546.1	447.2
Tl	537.3	439.3
Tl	535.1	437.8
Fe	527.1	430.6
Fe	522.7	426.5
Cu	521.8	425.4
Fe	519.2	423.1
Mg	518.4	422.6
Mg	517.3	420.9
Fe	516.9	420.5
Fe	515.3	418.8
Cu	510.6	414.1
Cd	508.6	411.7
Fe	505.1	408.2
Fe	500.6	403.1
Fe	492.0	392.7
Fe	485.9	386.6
Cd	480.0	377.0
Cd	467.8	359.3
Sr	460.8	347.5

Contribution to the Research on the Respiration of Fishes.

III. On the Change of the Alkali Reserve of Blood due to the Respiratory Condition in a Fish and some Marine Invertebrates.

By

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INTRODUCTION

The experiments presented in this paper have been designed, in the first place, to indicate the change in the alkali reserve of the blood of a fresh water fish and some marine invertebrates due to the change of the respiratory condition. In the second place, they were also aimed to show whether this change implies some biological significance in respect to the habitat or behaviour of these animals.

In a paper published ten years ago COLLIP (1920) stated the alkali reserve of marine fish and invertebrates and suggested the presence of a marked change in this quantity. POWER (1922) stated the alkali reserve of the blood of fish and pointed out the change of blood alkali due to the alteration of the tension of CO_2 as such. In a later publication (1928) he made a statement implying that the fish which rapidly changes its blood alkali is more resistant to the change of O_2 and CO_2 tension in breathing water. It is also of much interest to note the results of WINTERSTEIN's experiment (1921) which shows that the CO_2 content of the blood of Maja, a decapoda crustacea, changes exceedingly according as it breathes in water or in the atmospheric air. The present author likewise made studies on the oyster (1929) and fishes (1930), and indicated that the blood alkali changes with the respiratory condition. As for the same relation in higher

animals such as mammalia many works have been done, and results were summarized by HENDERSON (1928), VAN SLYKE (1926), and other authors.

In the present work the experiments have been conducted with the marine lower forms which have hitherto remained quite untouched in this regard. And the results were compared with those of the authors just cited.

METHODS.

The increase of alkali reserve in the blood has been tested under three conditions: (1) Animals were exposed to the atmospheric air by taking them out·of water; (2) Animals were forced to respire in the acidified sea water; (3) Animals were subjected to respiration under high CO_2 tension.

For the first purpose the specimens were simply placed on the floor of the laboratory, and were bled at convenient intervals. To meet the second view the pH of sea water was lowered by adding HCl in varying quantities, maintaining the pH as low as from 2.40 to 5.00. The quantity of breathing water was 2 to 5 litres, though it varied with the experiment. The container used in this case was an open glass jar measuring about 20 to 25 cm in diameter and 14 to 17 cm in depth. After submersing the animal into the acidified water the blood collections were made at appropriate intervals, taking out the animals one by one, and the water temperature and pH of water were also observed at these times. For the third purpose, i. e. in the experiment under high CO_2 tension, the animals were confined in a tightly closed glass jar of a little larger size than those used in the former cases. The stream of CO_2 gas was constantly bubbled through the water thus raising the tension of this gas even up to saturation in some cases.

The blood or coelomic fluid was collected from the heart in *Cyprinus* and *Arca*, and from the body cavity in *Caudina* and *Helio-cidaris*. In any of these animals 5 to 20 cc of blood was readily collected from an individual. In determining the hydrogen ion concentration on the blood of *Cyprinus*, the colorimetric method (1) was employed, diluting the blood 21 fold with a saline solution. With

regard to the other animals, 5 cc of fluid was delivered through a
syringe into a test tube under a paraffin oil, thus avoiding contact of
the blood with the atmospheric air. After adding 0.5 cc of an indicator
such as phenol sulphonphthalein or tetrabrommetacresol sulphonthalein,
the tube was centrifugalized in order to settle the red blood corpuscles.
Then the colour of the tube was compared to the colour standard
and its pH was determined. Consequently the protein or salt error
was disregarded.

In determining the CO_2 content or O_4 content of the blood or
coelomic fluid, the manometric blood gas apparatus of VAN SLYKE
(1924) was employed. The capacity of the extracting pipette was
50 cc. The quantity of blood used for each determination was 1 cc.
' S ', i. e. the volume of solution in the extracting chamber, was 3.5 cc.
The volume precent factor was taken from VAN SLYKE (1924) without
making any modification.

MATERIALS.

The animals employed for the present experiments were a fresh
water fish and three kinds of marine invertebrates: that is, *Cyprinus
carpio, Arca inflata (Anadra inflata)* a bivalve, and two species of
Echinodermata named *Heliocidaris crassispina* (sea-urchin) and *Caudina
chilensis*. Among these species *Cyprinus* is a common fresh water
fish, which has been invariably used since my first investigation (1927).
Arca is a large edible bivalve native to the muddy bottom of 20 to
30 meters or thereabouts, and is a vigorous variety, affording a large
amount of red blood for experimental purposes. The specimens used
were collected two weeks prior to the experiment. *Heliocidaris* is a
dweller in shallow water below the low tide mark and is well known
as the common sea urchin. Since this animal has a spacious body
cavity filled with transparent body fluid, it was very convenient for
the present study. The specimens employed were reared for 1 month
in the aquarium before use. As regards *Caudina chilensis*, it dwells
in the sandy shallow bottom near the tide marks, hence its habitat
is often exposed to the atmospheric air during low tide. This animal
has red blood in quantities, filling the coelomic cavity, and furnishes
fine material for blood investigation. Since this animal loses its natural

state in capitivity it was collected just before use.

Protocols of the Experiment.

On each species 2–4 experiments of different kinds have been carried out except one on which only the high CO_2 tension has been tested. The descriptions of the experimental conditions, which varied somewhat with the experiment, will be given below.

I. Cyprinus

Exp. 1. Breathing under high CO_2 tension.

The change of CO_2-content of the blood of this fish due to acidosis or alkalosis is already reported in my former publication (1). This time, therefore, only the influence of high CO_2 tension of breathing water upon the CO_2-content of blood has been examined. For this purpose three fishes were introduced to ten litres of water, after examining their normal blood pH and CO_2-content.

From the start of the experiment the CO_2 gas and air were bubbled through the water in order to raise the CO_2 tension and also to prevent the diminution of O_2 content in the water. This procedure served to keep the tension of CO_2 moderately high, thus avoiding the saturation which would raise the pH of the breathing water too high. The water temperature was carefully regulated so as to be maintained at 20° throughout the experiment.

The fish behaved normally under the above conditions for 20 minutes. After 25 minutes the fishes began to be in agony, and after 35 minutes they tended to jump about. From 45 minutes on they shook their heads convulsively, and one of them lay on the surface while the other fishes remained still, seeming very faint. The size of the fishes employed was as follows:

	Length (cm)	Height (cm)	Weight (gm)
Fish A	30.5	7.0	340
Fish B	30.5	6.8	330
Fish C	29.5	6.5	280

The data obtained are given in the following table.

TABLE 1.

Time after start	Water		Blood									Date Feb 1. 1930
	pH	Free CO_2 vol. %	pH				CO_2-content (vol. %)					
			A	B	C	Mean	A	B	C	Mean		
0 mns	6.40	—	7.50	7.50	7.60	**7.53**	30.80	28.31	29.01	**29.37**		11.00 A. M
5	5.40	10.50										
15	5.30	--										
25	5.20	32.83										
35	5.00											
45	5.00	38.57										
1 hr.	4.90	—	6.70	6.70	6.60	**6.67**	28.22	30.93	29.11	**29.42**		12.00 n

II. Arca.

Exp. 1. Exposure to air.

In this experiment a lot of shells consisting of 14 individuals were employed, continuing the experiment for a week. On the first day two of them were opened and bled as soon as they were taken out of the water, and thus the normal values of pH and CO_2-content were recorded. The remaining 12 shells were placed on the floor of the laboratory under room temperature, and two of them were examined

TABLE 2.

Duration of Exp.	Air temp	pH of blood			CO_2-content of blood. vol. %			Dimension of shells						Date (1929)
		A	B	Mean	A	B	Mean	Length (cm)		Breadth (cm)		Weight (gms)		
								A	B	A	B	A	B	
1st day	22°.0	7.90	7.80	**7.88**	5.58	6.92	**6.92**	12.0	13.0	9.0	9.0	350	440	June 26, 2.00 P.M
2nd ,,	23°.0	7.70	7.50	**7.60**	10.70	8.50	**8.60**	11.0	10.5	9.0	7.0	330	210	,, 27, ,,
3rd ,,	21°.5	7.50	7.25	**7.38**	9.94	11.20	**10.60**	10.5	11.3	8.0	8.3	180	240	,, 28, ,,
4th ,,	22°.0	7.50	7.20	**7.35**	11.20	10.30	**10.80**	12.5	11.0	9.2	8.0	350	260	,, 29, ,,
5th ,,	21°.0	7.10	7.60	**7.35**	13.40	11.70	**12.50**	12.5	11.0	8.5	5.7	2.265	195	,, 30, ,,
6th ,,	21°.5	7.40	6.70	**7.05**	8.70	16.00	**12.40**	11.0	12.5	8.0	9.0	220	230	July 1, ,,
7th ,,	23°.0	6.70	6.80	**6.75**	13.10	14.70	**13.90**	11.5	9.5	8.5	7.0	220	150	,, 2, ,,

every day. On the second day all the shells opened widely and the animals dangled their feet, but quickly closed when agitated. Such a state continued until the forth day. On the fifth day, however, two of the specimens, tightly closed their shells, but the others remained open as hitherto observed. On the sixth day all the shells closed. On the seventh day the animals seemed to be almost dying, and the individual which opened its shell responded only slightly to a strong agitation, continuing the heart beat but slightly. Observations are given, tabulated as Table 2.

Exp. 2.   Exposure to acidified water.

The breathing water used for the present experiment was the tap water of the laboratory, which showed its density to be 1,0250 (12°.5), and its proper pH was lowered to 5.00 (Exp. a) or to 3.40 (Exp. b) by adding hydrochloric acid   Two or three individuals were placed in five litres of this water in an open jar, and the water temperature was kept a little higher than 20° in both experiments. For the pur-

TABLE   3.

Time	Condition	Water temp	Water pH	pH	Blood CO_2 content vol %	Blood O_2 content vol %	Length (cm)	Weight (gms)
				Experiment a				
Start	Normal	10°.8	8.13	7.85	6.60	1.71	13.0	350
		10°.8	8.13	7.90	7.14	1.46	12.5	345
(Mean)		10°.8	8.13	7.88	6.87	1.59	—	—
6 hours later	Experimental	20°.7	6.00	6.70	10.51	1.11	13.5	400
		20°.7	6.00	6.70	9.11	1.22	13.5	395
(Mean)		20°.7	6.00	6.70	9.81	1.17	—	—
				Experimental b.				
5 hours later		25°.5	3.60	6.90	11.44	0.40	13.5	370
5.5 hours later	Experimental	25°.5	3.60	6.80	12.01	0.25	13.5	380
6 hours later		25°.5	3.70	6.80	10.16	0.01	13.0	365
(Mean)		25°.5	3.60	6.80	11.20	0.22	—	—

pose of comparison, however, two individuals which were kept under normal conditions were also examined. The alkalinity of sea water which showed about 40.0 cc in natural condition, was decreased to 24.0 cc (Exp. a)–20.2 cc (Exp. b). The oxygen content of sea water at the start was about 5.9 cc in both experiments.

When the animals were introduced into the acidified water they closed their shells at first. But from 30 minutes to 1 hour later they began to open and made opening and closing movements slowly.

Exp. 3. CO_2 saturating experiment.

The pH of sea water which was used for the present experiment showed 8.10 at the start, but it decreased to pH 5.80 at 14' after CO_2 gas had bubbled for one hour through the water. Thereafter the pH gradually decreased, giving 5.60 after 1.5 hours, 5.50 after 3 hours, and 5.30 after four hours. The CO_2 content of the sea water which showed 3.9 vol. % increased up to 27.50 vol. % by one hour later. The O_2 content which was determined to be 6.8 cc per 1. in normal condition decreased to 1.6 cc on account of the bubbling of pure CO_2. The water temperature ranged between 13.7–14.0 during the experiment.

Two individuals were placed in five litres of water in a closed vessel under the above conditions, continuing the bubbling of CO_2 until the end of the experiment. On the other hand two specimens which were kept under the same conditions were employed for the purpose of control.

As soon as the animals were introduced into the experiment, however, condition they closed their shells for a while. One of them, tes later, opened its shell after 13 minutes, and the other 30 ry. But after thenceforth making opening and closing movements e one keeping its 3 hours they seemed to have been narcotized movement after this. shell opened and the other closed, showing 6 hours, the heart beat When the determinations were made active and was on the verage of was observed to have become very ceasing.

TABLE 4.

Time	Material	Water temp	Water pH	pH	Blood CO₂-content vol %	Blood O₂-content vol %	Size of animal Length (cm)	Size of animal Weight (gms)
	Normal specimen	14° 0	8.10	7 80	5.01	1.82	13.5	440
Start		14°.0	8.10	7.75	6.34	2.01	12.5	350
	(Mean)	14° 0	8.10	**7.78**	**5.68**	**1.92**	—	—
	Exp specimen	13° 7	5.30	6.20	20.79	1.27	12.5	360
After 6 hours		13°.7	5.30	6.30	23 90	0.45	12.0	300
	(Mean)	13°.7	5.30	**6.25**	**22.35**	**0.86**		

III. Heliocidaris.

Exp. 1. Normal pH and CO₂-content of coelomic fluid.

By inserting a syringe needle into the body cavity through the peristomial area, five or more cc of body fluid can be collected from an animal of normal size. This fluid contains many amoeboid corpuscles and bears a resemblance to the fluid of their water vascular system which also contains the corpuscles. As this fluid changes its pH rapidly the treatment had to be made very quickly. From the twenty determinations thus made the following results were obtained.

TABLE 5.

Case No	Air ,,	Water temp	Coelomic fluid pH	Coelomic fluid CO₂-content vol %	Size of specimen Diameter (cm)	Size of specimen Weight (gms)	Date (1929)
1	28°.0	27°.0	7.50	6.89	5.3	51.5	Aug 14, 7–30 a.m.
2	29°.0		7.45	5.65	5.0	42.0	Aug. 15, 2–30 p.m.
3	30°.3		7. ,	6.02	5.2	45.0	,,	3–00
4	30°.5		7.40	5.34	4.9	43.0	,,	3–30
5	30°.6		7.55	5. ,	5.8	71.0	,,	4–00
6	28°.0	25°.4	7.72	7.24	4.6	40.0	Aug 16, 7–00 a.m.
7	28°.0		7.50	6.99	5 7	35.0	,,	7–30
8	28°.1		7.60	5.81	4.7	37.0	,,	8–00

Case No.	Air temp	Water temp.	Coelomic fluid		Size of specimen		Date 1929
			pH	CO_2 content vol. %	Diameter (cm)	Weight (gms)	
9	28°.2	25°.4	7.70	6.66	4.8	38.0	Aug 16, 8-30 a m
10	29°.0	25°.5	7.70	5.38	4.4	40.0	,, 9-00
11	29°.5	26°.0	7.90	5.70	4.3	37.5	,, 11-00
12	30°.0	26°.0	7.70	5.09	4.1	33.0	,, 11-30
13	30°.0	26°.2	7.50	6.15	3.8	27.5	,, 1 30 p.m.
14	30°.0	,,	7.65	5.36	4.1	32.0	,, 2-00
15	29°.3	,,	7.80	6 68	4.0	31.0	,, 2-30
16	29°.3	,,	7.80	7.61	5.0	46.0	,, 3-00
17	29°.3	,,	7.82	6 86	4.0	28.0	,, 3-30
18	29° 3	,,	7.45	5 64	4.0	30.5	,, 4-00
19	29°.3	,,	7.80	6.20	4.0	28.0	,, 4-30
20	29°.3	,,	7.60	5.56	4.3	33.0	,, 5-00
Mean		---	7.68	6.10	---	-	

Exp. 2. Exposure to air.

In order to keep the animals exposed to the atmospheric air, several specimens which were taken from the water were placed from an empty glass jar. The body fluid were collected from these animals at intervals of from 1 to 4 hours. Under such experimental conditions they never protruded the tube feet, but moved slowly only by spines. This animal is much weaker than *Arca* for air exposure, and most of them ceased to move within 10 hours, so that the experiment was finished in 8 hours. Results obtained were as follows.

TABLE 6.

Case No.	Time (hrs)	Air temp.	Body fluid			Size of animal		Date (1929)
			pH	CO_2 content vol. %	O_2 content vol. %	Diameter	Weight	
1	Start	22°.0	7.50	4.70	0.35	5.0	60	Sept. 26, 8-00 a m
2	1	22°.0	7.30	4.80	0.30	4.0	39	,, 9-00
3	2	22°.0	7.30	5.01	0.31	4.5	38	,, 10-00
4	4	23°.0	7.20	5.54	0.21	4.5	40	,, 12-00
5	8	23°.0	7.10	6.45	0.10	4.3	30	,, 4-00 p.m

Exp. 3.. Exposure to acidified water.

The pH of breathing water was lowered to 2.40 by adding HCl solution. Five animals were introduced into the 2 litres of this water in an open jar. Soon after the introduction they ceased to move; the tube feet remained shortened and bent, thus showing an indication that the acidity of this degree affects the animals very badly. Thirty minutes later small bubbles evolved from the surface of the shell and spines. By replacing one of them in normal sea water after one hour I found that they quite recovered and became active. During the experiment the water was not renewed, and five observations were made.

TABLE 7.

Case No.	Time after start (hrs)	Air temp	Water Temp.	Water pH	Body fluid pH	Body fluid CO_2 content vol. %	Size of specimen Diameter (cm)	Weight (gms)	Date (1929)
1	Start	28°.0	22°.5	2.40	7.50	6.00	3.7	37	Aug. 18, 10-00 a m
2	1	28°.5	22°.8	—	6.20	11.30	3.8	36	„ 11-00
3	2	29°.0	24°.0		5.80	16.65	4.2	37	„ 12-00 n
4	4	29°.0	24°.0	—	5.40	20.35	4.5	39	„ 2-00 p.m.
5	8	26°.0	23°.0	4.20	5.40	22.10	4.1	38	„ 6-00 p.m.

Exp. 4. CO_2 saturating experiment.

Five individuals were confined in a closed jar containing 4 litres of water, and the stream of CO_2 gas was bubbled through the water until the end of the experiment. After 4.5–5.5 hours the body fluid was collected and tested.

Though the normal pH of sea water was 8.18 at the beginning, it lowered rapidly with the introduction of CO_2, giving 5.60 after 10 minutes, and showed after this 5.30, 5.15, and 5.10 after 1, 2, and 3 hours respectively. The CO_2 content of sea water increased from 4.16 vol. % to 67.8 vol. % due to the solution of CO_2 gas.

TABLE 8.

Case No.	Time after start (hrs)	Water		Body fluid				Size of animal	
		Temp.	pH	pH	Total CO₂ content. vol. %	BHCO₃ vol %	Free CO₂ vol %	Diameter (cm)	Weight (gms)
1	4°.0	21°.3	5.10	6.30	61.63	50.15	11.48	4.7	40
2	5°.0	21°.2	5.20	6.20	78.03	52.51	25.52	3.8	30
3	5°.5	20°.5	5.20	5.90	69.13	51.90	17.23	3.7	33
Mean	—		5.20	6.19	69.60	51.52	18.04	—	—

IV. *Caudina.*

Exp. 1. Exposure to air.

Similar to the same experiments made on other animals, several individuals were exposed to the atmospheric air, by placing them in an empty open jar. The blood collection was made at the interval of 1-2 hours, employing different individuals for each single determination, thus using 35 individuals in all. The pH of the blood was determined by diluting it 6 fold by a saline solution. The size of the animals used measured from 11 to 19.5 cm in diameter and from 25 to 32 gms in weight.

TABLE 9.

Case No.	Time after start (hrs)	Air temp	pH of blood			CO₂-content			
			Exp. 1	Exp. 2	Mean	Exp. 1	Exp. 2	Exp. 3	Mean
1	0	24°.0	7.60	7.65	7.63	8.40	8.20	8.40	8.33
2	1	24°.0	7.35	7.32	7.33	8.30	8.10	8.40	8.37
3	2	24°.3	7.25	7.20	7.23	9.00	9.40	9.02	9.14
4	3	24°.6	7.40	7.50	7.45	8.95	10.30	9.47	9.57
5	4	24°.5	7.40	7.10	7.25	9.58	9.70	9.21	9.53
6	5	24°.3	7.10	7.50	7.30	11.50	8.89	9.63	10.00
7	6	24°.2	7.10	7.50	7.30	11.30	10.30	12.2	11.30

Exp. 2. Exposure to acidified water.

At the time of the experiment the tap water showed its pH to be 8.20, the temperature to be 12°.8, and the specific gravity to be 1.0251(12°.8). To meet the purpose of this experiment the pH was lowered to 3.50 by adding HCl, and the temperature was raised to 20°.0 with a view to accelerating the effect of the low pH. Five animals were admitted to five litres of the water in an open jar.

From the outset of the experiment the pH of the water tended to rise, so that the occasional addition of HCl was necessitated to keep the pH low. After three hours time the determination of pH and CO₂-content was made every hour, making five blood collections. The O₂ content of the water was 5.67 cc per 1. at the start but it decreased to 4.33 cc due to the respiration of the animals. *Caudina* survived vigourously throughout the experiment notwithstanding the expectation that such high acidity might affect them very badly.

TABLE 10.

Case No.	Time after start (hrs)	Water			Blood			Size of animal	
		Temp	pH	pH	CO₂-content vol % BHCO₃	Free CO₂	O₂ content vol. %	Diameter (cm)	Weight (gms)
1	3	19°.7	3.80	4.70	13.21	0.26	1.37	3.5	71
2	4	20°.0	3.60	5.20	13.13	0.13	0.52	3.3	55
3	5	20°.0	3.80	5.10	17.16	—	0.11	3.1	60
4	6	20°.0	3.90	5.40	14.63	—	0.13	3.5	98
5	7	19°.5	3.90	5.00	15.63		0.10	4.0	82
Mean	--	--	3.80	5.08	14.75	0.20	0.45	—	—

Exp. 9. CO₂ saturating experiment.

The condition of the water at the start of the experiment was almost the same as in the foregoing experiment. As soon as the 6 individuals were introduced into the 5 litres of this water in a closed vessel, the stream of CO₂ gas was passed through till the experiment was finished.

The animal appeared normal, moving their tails for 30 minutes.

During the next 30 minutes, however, the animals seemed to have been narcotized, as movement almost ceased. After three hours the animals were much weakened, showing no tail movement. But at the time of bleeding they responded markedly to the stimulus of the syringe needle, and strongly contracted their bodies.

The CO_2-content of sea water, which was 4.01 cc per 1. in normal conditions, increased to 66.06 vol. %, and the O_2 content which was 5.39 cc per 1. decreased to 0.23 cc by the end of the experiment. The increase of CO_2 in this case might be due, for the most part, to the solution of free CO_2, and the decrease of O_2 might partly be due to the respiration of the animals and partly to the expulsion of O_2 due to the stream of pure CO_2. The pH of the water, which was maintained at 8.20 at the beginning, lowered to 5.80 after 10 minutes, and it further lowered afterwards, giving pH 5.20, 5.10, 5.00, and 4.90 after 1, 2, 3, and 5 hours respectively. The following table represents the results.

TABLE 11.

Case No.	Time after start (hrs)	Speci-men	Water		Blood				Size of animal	
			Temp.	pH	pH	CO_2-content vol. %		O_2 content vol. %	Dia-meter (cm)	Weight (gms)
						BHCO$_3$	Free CO_2			
1	0	Normal	11°.8	8.20	7.60	9.49	—	1.09	3.1	65
2	0		11°.8	8.20	7.80	9.31	—	1.54	2.9	70
3	0		11°.8	8.20	7.55	9.68	—	1.09	3.4	71
4	0		11°.8	8.20	7.56	8.88	—	1.81	2.8	63
5	0		11°.8	8.20	7.60	8.95	—	1.21	3.0	69
Mean	—		—	8.20	7.62	9.26	—	1.35	—	—
6	3	Experimental	21°.5	5.10	5.20	45.37	16.09	0.13	3.4	75
7	4		20°.5	5.20	5.20	59.35	24.07	0.15	3.2	69
8	5		19°.6	5.20	4.90	46.19	30.36	0.25	3.3	70
9	6		19°.6	5.25	4.90	36.51	40.20	0.09	3.3	75
10	7		19°.5	5.25	4.90	47.03	24.16	0.11	3.2	62
Mean	—		—	5.19	4.96	46.89	26.96	0.15	—	—

Results and Discussion.

The blood alkali is one of the important factors concerned with the regulation of respiration. This is not only because the blood alkali determines the CO_2 carrying power of the blood but also because it relates to the dissociation of oxygen from hemoglobin as it regulates the pH of the blood in association with the free CO_2 in the blood.

POWER (13) made a study on fish and indicated that the alkali reserve of the plasma of the blood of the viviparous perch is increased by a high CO_2 tension and decreased by a low CO_2 tension of the medium, seemingly independent of the pH as such. In the writer's work (3) the absolute change of alkali in the whole blood was reported, and the same is the object of the present experiment.

In the present work the increase of blood alkali was studied under three conditions, i. e. (1) Exposure to air, (2) Breathing in acidified water, (3) Breathing under high CO_2 tension. Of these conditions exposure to air produces oxygen deficiency upon most aquatic animals. In the higher animals in which the regulating mechanism of respiration is developed enough, the oxygen deficiency excites the breathing unfailingly. As a result of this excitation an excessive amount of CO_2 evaporates from the blood and decreases the alveolar CO_2 tension and consequently the arterial CO_2 tension. And hence the blood pH rises, due to a temporal alkalosis until an adequate amount of alkali can escape from the blood. But with the elimination of alkali this alkalosis recovers and the blood pH returns to the normal level. The lower animals, however, seem to have no such regulative power. The O_2 deficiency stimulates the breathing but in an inappreciable degree. But the change of blood pH due to the accumulation of H_2CO_3 is regulated by the neutralizing effect of the bicarbonate or carbonate which is furnished as spicules, shells, etc. The deficiency of oxygen, on the other hand, could be endured by making anerobic respiration as is found in the Oyster (KOKUBO, 2). On the blood condition under O_2 deficiency in the Oyster, I reported that the blood alkali increases and the pH falls. From these phenomena I infer that these processes must be a regulation to endure the temporary cessation of respiration during low tide. If this be the case the fact that the increase of alkali varies according to species (COLLIP, 8) may be

accounted for as being due to the difference in ability of acclimatiza-
tion to environment. To demonstrate whether this opinion hold true,
experiments were made on animals which are nearly related in
zoological order to each other, but differ in behaviour.

For the purpose of comparison with the studies on the Oyster
(2), I made, firstly, an experiment on *Arca* which is an inhabitant of
muddy bottoms and has no fear of being exposed to atmospheric air.
As can be seen from Table 1, the blood alkali of this animal also
increases and the blood pH falls very regularly with time during the
week's experiment. The amount of blood alkali nearly doubled in
seven days. Such a rate of increase, however, is incomparably small
as contrasted with that of the Oyster, in which it increases as much
as over nine fold in the same duration of time. The blood pH in
Arca showed a striking fall in a week's time, decreasing from 7.88
to 6.70, and showed a far greater rate than that of the Oyster. Of
the two characters, the blood alkali and blood pH, the increase of
the former favours the life condition as it hinders the fall of blood pH
while the abnormal fall of blood pH affects the animal unfavourably.
Therefore, it may be said that *Arca* is less furnished with the ability
of defending itself against this unfavourable condition due to the
temporary cessation of respiration. These facts are consistent with
the idea that the Oyster must be better adapted for the same condition,
as it is habitually exposed to the atmospheric air during low tide.
While as to *Arca*, it has not so serious a need of this ability as its
habitat is never exposed to the atmospheric air, and this above
mentioned ability seems to suffice the need of alkali in enduring the
temporary cessation of respiration which occurs, if ever, through causes
other than exposure to air.

With regard to the *Heliocidaris* (sea urchin), which I have not
hitherto investigated in this regard, the normal pH and CO_2-content
of the body fluid were determined first of all. As will be seen from
Table 4, the mean value of twenty determinations showed the normal
pH to be 7.63 and the CO_2-content to be 6.10 vol. %. This indicates
that the pH of the body fluid is much lower than that of the sea
water, in spite of the fact that its alkali reserve (CO_2-content) is
decidedly higher than that in sea water. Consequently it can be said
that the body fluid of a sea urchin is better buffered than sea water.

A comparison of these values in several marine forms (KOKUBO, '27, '29) shows the following results:

	pH	CO_2-content (vol. %)
Heliocidaris (sea urchin)	7.63	6.10
Caudina (sea cucumber)	7.79	8.50
Arca (bivalve)	7.75	6.30
Ostrea (oyster)	7.39	4.22

On exposing Heliocidaris to the atmospheric air I have discovered that (Table 5) the alkali reserve increases and the pH falls with time as is the case with Arca. In this case, however, the increase of the alkali reserve was but faint giving a rate of about 1.4 fold in eight hours time; while the pH rise was pretty marked, as will be seen in Table 5. The oxygen content which was determined along with the other factors showed a decreasing tendency with the course of time.

That this animal might be less adapted to air exposure was to be expected from its faintness during the experiment. This anticipation was realized as the increase of the alkali reserve was found to be only a little.

Another experiment along the same line was made on Caudina.

In Caudina the result of exposure to air was rather irregular and indistinct. The pH of the blood gradually decreased during the first two hours. But from this time on the change became irregular and the pH differed greatly according to the individual. The alkali reserve showed a general tendency to increase with time, except for the small discrepancies which seem to be due to individual differences. In addition, after throughly exammining these data we note that the increase in this case is not a distinct one, because the range of these changes exceeds the limit of normal range (KOKUBO, 1927) but a little. Although the change was thus unmarked we may possibly infer from the present result that in Caudina also the blood pH falls and the alkali reserve increases due to the exposure to air.

When compared with Heliocidaris, this animal lives in shallower water and sometimes dwells beyond low tide mark. Consequently, it often has to cease normal respiration during low tide. In this connection it might need more alkali reserve than Heliocidaris. It is probable, however, that Caudina may respire through the body skin, as was

stated by WINTERSTEIN (1921) with regard to some *Holothuroidea*. The circulation of coelomic blood in *Caudina* (YAZAKI, 1930) seems to suggest the possibility of skin respiration in this animal. If such be the case, the reason why this animal has less alkali reserve than *Heliocidaris*, which is not adapted for exposure to air, can readily be recognized.

Having thus discussed the experiment on exposure to air, we may now pass on to the results of the experiments made by exposing the animal to acidified water. In my former paper I reported that in the Oyster the H-ion penetrating from breathing water into the blood increases the blood bicarbonate at a great rate. This is seemingly contrary to the same case of the blood of higher animals in which invasion of the H-ion liberates the CO_2 from bicarbonate and decreases the blood alkali. This apparent discrepancy is, however, granted by conceiving that the H ion which invaded into the circulatory system of the Oyster firstly neutralizes the blood bicarbonate, but then it dissolves the shell carbonate, evolving CO_2, and in the presence of this CO_2, the shell carbonate becomes calcium bicarbonate. Therefore we may say that the H ion, i. e. acid, in this case might serve in producing CO_2, and in virtue of this CO_2 the blood bicarbonate is increased. To see whether this is also the case with other forms and to see what difference could be found if the materials varied, several experiments have been made.

Arca lowers its blood pH markedly by breathing in acidified water for five or more hours. Table 2 shows the lowest value attained to be 6.70 in Exp. a, and 6.80 in Exp. b. The fact that the blood pH fell lower in Exp. a, in which the water pH was held comparatively higher, may be due to the difference of behaviour showed by individuals. In Exp. b, the animal closed its shell for a long while due to the stimulus of the high acidity of the water, so that the blood pH may have been kept a little higher than in Exp. a.

The CO_2 content increased in either of these two experiments up to 1.7 to 2.0 fold of the initial content. The O_2 content, on the other hand, showed a decreasing tendency. In short, these results agreed with what was found in the Oyster (2).

The results obtained on *Heliocidaris* (Table 6) followed closely those of *Arca*. The CO_2-content, which maintained a normal value

at the start, became about two fold as much as the initial value in one hour's time. After this it increased more and more, attaining to 22.1 vol. % ; that is, about 3.7 fold of the normal content, at the end of the experiment. The pH of the coelomic fluid lowered with time, showing 6.20 and 5.80 in 1 and 2 hour's time respectively. Thereafter the lowering became slow and showed 5.40 after 4 hours, indicating no more rise thenceforth. Comparing these results with those of *Arca* one will note that both the change of pH and CO_2-content is much more marked in *Heliocidaris* than in *Arca*. The speedy rise of the CO_2-content of the coelomic fluid may be considered as due to the rapid solubility of the shell carbonate.

As for *Caudina* (Table 9) the increase of CO_2-content was less marked than in *Heliocidaris* and showed a rough approximation to that of *Arca*. It seems to be noteworthy that in this animal the increase of blood alkali was for the most part accomplished in three hours time, and after this, the increase became somewhat irregular. The oxygen content of the blood decreased with the course of time in contrast to the change of CO_2-content, and showed a parallel relation to the pH change. The lowering of blood pH was also very marked within three hours, and thereafter the change became a little irregular.

In short, any of these three invertebrates increases the alkali, and decreases the pH and O_2-content by breathing in acidified water for several hours. The increase of alkali in this case may be thought to be due to the bicarbonate which is formed by the action of CO_2 upon calcium carbonate. For the CO_2, the action of HCl upon calcium carbonate may be responsible.

With regard to the experiment which was made under high CO_2 tension, the increase of blood alkali in invertebrates was previously expected from what was found on the Oyster (2). But on *Cyprinus* the problem of blood alkali under the high CO_2 tension may become more intricate. Within the physiological range of CO_2 tension, the blood alkali will increase with the rise of CO_2 tension, as can be understood from the ordinary CO_2 dissociation curve. According to Y. HENDERSON's (14) experiments which were made on dogs' blood, the amount of alkali produced in the whole blood reaches its maximum somewhat below 440 mm.

From the above conception, I expected that the blood alkali of

Cyprinus might be increased to some extent if the CO_2 tension of water should be raised. But contrary to this expectation there has not been found any increase in blood alkali, as can be seen in Table 1. In this regard I am reminded of HALDANE's (17) statement that a several hours' stay of man in air containing 5 to 6 percent of CO_2 was also not sufficient to increase appreciably the available alkali of the blood, although the titration acidity of the urine was increased. This means that even unusually high tension of CO_2 is incapable of increasing blood alkali on account of the regulative power of respiration. The presumption that such might also be the case with the present investigation may be elicited from the results given in Table 1.

From Table 1, one will note that the pH of the blood of *Cyprinus* decreases with the pH of water, attaining at last to 6.67 in mean value. As to the CO_2-content, however, there has not been found any appreciable change in mean value, although each individual value showed some minor increase or decrease. This signifies that the blood shows acidosis in which the pH is lowered but the alkali remains unchanged. But at any rate the fact that the CO_2-content does not increase in spite of rise of CO_2 tension is seemingly contrary to the general rule which tells us that a rise of CO_2 tension changes the distribution of sodium between cell and plasma and consequently increase the CO_2-content. The question whether this immobility of blood alkali has some connection with the regulative power of respiration, may only be solved by a further detailed investigation

Contrasted with the results obtained on *Cyprinus*, the effect of high CO_2 tension upon the blood alkali of *Arca* has been found to be very marked. As is seen in Table 4, the blood pH fell markedly within 6 hours, showing a mean value of pH 6.25. The CO_2-content, on the other hand, increased remarkably, attaining at last about 3.9 fold of the normal value. As has been repeatedly stated, such a prominent increase of CO_2-content may doubtlessly be attributed to the bicarbonate generated from the shell carbonate. In contradiction to the increase of CO_2-content, the O_2 content of blood decreased very markedly, giving about a half of the normal value at the end of the experiment. Such decrease, however, may reasonably be explained by understanding that the oxygen dissociation curve of the blood flattens as the CO_2 tension increases.

In *Heliocidaris* also the effect of the high CO_2 tension was very marked, and showed a similar tendency as in the case of *Arca*. The pH of the coelomic fluid fell with time, giving 5.90 at the end. The bicarbonate of the coelomic fluid increased up to 51.5 vol. % in mean; i. e., about 8.6 fold of the normal value. As can be seen in Table 8, free CO_2 also increased, occupying about 27% of the total CO_2-content.

The effect of the high CO_2 tension upon the blood alkali of *Caudina* was almost similar to the preceding two species in general trend. The blood pH lowered to 4.90 in seven hours time. The bicarbonate content increased at a great rate, attaining at last about five fold of the normal value in mean. In comparison to *Heliocidaris*, the increase of free CO_2 was much more marked, giving 26.98 vol. % ; i. e., about 37 % of the total CO_2-content. The oxygen content of the blood decreased as in the case of *Arca*, probably according to the effect of CO_2 tension upon the oxygen dissociation curve of the blood.

Looking through all the experiments, except in *Cyprinus* in which a quite different result was obtained, we can conclude that any of the three experimental conditions; i. e., (1) Exposure to air, (2) Exposure to acidified water, (3) High CO_2 tension, increases the alkali reserve of the blood or coelomic fluid. Of these three cases, however, the high CO_2 tension was most effective, and air exposure was most inefficient. On *Heliocidaris* and *Caudina* a marked increase of free CO_2 in the blood due to high CO_2 tension was observed, but in the case of acidified water such increase of free CO_2 seems to be very slight. In contrast to the increase of alkali, the O_2 content reduced decidedly by the same causes. This hints that the low pH of the blood flattens the oxygen dissociation curve of the blood. As regards the blood pH, it was observed that these three experimental conditions equally lowered the pH, though the lowering due to high CO_2 tension was by far more rapid than that caused by the acidified water or by exposure to the air.

SUMMARY

1) The change of the alkali reserve of blood due to respiratory conditions in fish and marine invertebrates has been investigated.

2) In *Cyprinus* there has been found no change in alkali reserve due to respiration in water of high CO_2 tension.

3) *Arca, Heliocidaris*, and *Caudina* markedly increase the alkali reserve of the blood or coelomic fluid by breathing in water of high CO_2 tension or acidified water, or as they were exposed to the atmospheric air.

4) The rate of the increase of alkali reserve was the greatest under high CO_2 tension and smallest under exposure to the air.

5) The pH of the blood or coelomic fluid was lowered by any of the above experimental conditions.

6) With the lowering of the pH of the blood or coelomic fluid the oxygen content of these fluids decreased decidedly.

7) Among these invertebrates studied, the increase of alkali reserve due to exposure to the air was most remarkable in *Arca* and least in *Caudina*.

8) The increase of blood alkali, within 7 days, in *Arca* (2 fold) due to the exposure to air is much less than that observed in the Oyster (9 fold). This seems to suggest that the Oyster is better adapted for the exposure to air than *Arca*, as the natural habitat of the former is often exposed to atmospheric air, while that of the latter is never exposed.

The author takes this opportunity of expressing his hearty thanks to Prof. S. HATAI for his kindness in criticizing the results of the present investigation.

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Studien über die Leuchtsymbiose in *Physiculus japonicus* Hilgendorf, mit der Beilage der zwei neuen Arten der Leuchtbakterien.[1]

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(Mit 4 Tafeln und 3 Text-figuren)

INHALT

I   EINLEITUNG

Die Geschichte der Leuchtsymbiose der Fische ist überhaupt eine recht kurze. Als Erster erkannte HARVEY 1921 die Einschlüsse in den geöffneten Leuchtorganen von zwei Arten der in Atollen der Bandainseln lebende Fische, *Anomalops* und *Photoblephalon*, als Leuchtbakterien an. Es gelungen ihm, die Bakterien auf künstlichen Nährböden zu züchten. Aber weil die Kulturen nicht geleuchtet waren, bezeichnete man die Entdeckung HARVEYs noch bis vor einigen Jahren als eine nicht unzweifelhafte Tatsache, aber dass seine Angabe richtig war, wurde später (1928) durch von YASAKI gemachte Arbeit über die *Monocentris japonicus* (HOUTTUYN) mitterbar bestätigt. Durch eine bakteriologische Untersuchung hat YASAKI in den Leuchtorganen

dieser Fischart die symbiontischen Leuchtbakterien gefunden. Da
Anomalops und *Photoblephalon* eigentlich nebst *Monocentris* syste-
matisch die von derselben Gruppe sind, und zwar ihre Leuchtorganen
sehr gleich gebildet sind, ist es sehr schwierig, bei jeder der oben
genannten drei Fischarten die Lichtproduktion ganz verschieden zu
bemerken.

    Physiculus japonicus HILGENDORF, zu *Gadidae* gehörig, lebt überall
in Seen nahe der Küsten von der nördlichen Hälfte des Zentral
Japans. Franz, der eine ausführliche Angabe über diese Fischart
gemacht, erregte sich eine Aufmerksamkeit auf eine am Bauche be-
findliche, schuppenfreie, schwarzgefärbte Scheibe und fand unter der-
selben im Muskelfleisch eine schöne Drüse aber äusserte er sich nur
mit den Worten, dass „ich keine Vermutung aufstellen kann."

    Nun ist doch die obige Frage mit dieser meiner Arbeit zur Lösung
gebracht. Die Drüse ist denn tatsächlich ein Leuchtorgan, die in sich
symbiontische Leuchtbakterien enthält. Hier ist über dem Leuchtorgan
von *Physiculus japonicus* sowie über zwei neue Arten von Leucht-
bakterien, die bei diesen Untersuchung gefunden wurden, berichtet.

    Im Gegensatz zu dem Leuchtorgan der *Physiculus japonicus* in-
teressiert mich die Resultat des Versuches HICKLINGS über dem
Leuchtorgan der *Malacocephalus laevis*, dass das Organ keine Leucht-
bakterien enthält, sondern eine Leuchtsubstanz ausscheidet. Auf dem
Kicklings Bilde ist das Leuchtorgan der letzteren fast in derselben
Stelle und von derselben Struktur wie bei der ersteren. Wäre es
nicht wunderbar, dass die Organen dieser zwei so naheverwandten
Fischarten, die sogar von gleichen Strukturen sind, sich nur in der
Lichtproduktion ganz verschieden verhalten?

II. TIERMATERIAL.

    Das Tiermaterial in dieser Untersuchung wurde in November 1929
aus dem Golf „Mutsu" herausgeangelt, und war noch eine Woche
im Aquarium im Asamushi Marine-Laboratorium für Biologie lebend
gelassen, inzwischen wurde bei diesen lebenden Fischen beobachtet, ob
sie wirklich aus ihren Leuchtorganen ausstrahlten, und dann Züchtung
der Leuchtbakterien aus dem Leuchtorgan u. a., Fixierung der Organen
für Schnittenpräparate und alles, was nur bei lebenden Fischen gemacht
werden könnte, wurden ganz ausgeführt.

III. LEUCHTORGAN.

Wie schon in Einleitung erwähnt wurde, ist die Fischart charac-
terisiert durch die kleine, an Bauche gelegene, schuppenfreie, schwarze,
runde Scheibe, die vor dem After in der Medianlinie liegt. Wenn
die Bauchwand an der Scheibe geöffnet wird, findet man im Muskel-
fleisch derselben eine schöne herzförmige Drüse eingebetet. Umgeben
ist die Drüse von einer harten Kapsel, wo sich schichtweise massen-
hafte Chromatophoren befinden. Ein Ausführungsgang verläuft von
dem Hinterteile der Drüse aus durch das Muskelfleisch der Bauchwand,
um endlich im Rektum, nahe dem After zu münden. Das Leuchtorgan
von *Physiculus japonicus* besteht aus den drei Bestandteilen: die
Scheibe, die Drüse und ihr Ausführungsgang. Die Grösse der Scheibe
und die der Drüse sind je nach der Grösse des Fisches voneinander
abweichend, aber dieses Verhältnis bricht zugunsten der kleinen Fischen
ab, z. B. die Scheibe des Specimens von 37 cm besitzt das Durchmesser
von 4.0 mm, die des Specimens von 15 cm aber das von 2.5 mm, das
Erstere hat die Drüse von der Länge 6 mm, das Letztere dagegen
die von der Länge 4.0 mm.

Feiner Bau des Leuchtorgans.

Für Schnittenpräparate wurde das Leuchtorgan mit dem aus See-
wasser hergestellten BOUIN'schen Gemisch fixiert. Das Stückchen
wurde im Paraffin eingebettet und in der Dicke von 4–8 μ geschnitten.
Diese wurden durch die Dopperfärbung, wo z. B. HEIDENHAIN'sche
Hämatoxylin-Orange G oder DELAFIELD'sche Hämatoxylin-Eosin benutzt
werden, gefärbt, aber besonders zur Demonstration der Bakterien in
der Drüse kamen dazu einige bakteriologische Färbungsmittel.

Die Drüse besteht hauptsächlich aus radiär gestreckten Schläuchen,
die sich je nach der Stelle verschieden entfaltet finden; sie sind am
besten an den ventralen und hinteren Regionen der Drüse entwickelt
und am Rücken, relativ weites Luhmen umfassend, aber am wenigsten.
Die Wandung des Schläuches besteht aus einer einfachen Schicht von
kubischen Drüsenzellen, mit Ausnahme von der Wandung des Fundus,
die mit gehäufter Zellen verdickt ist.

Der Wand des Schläuches entlang, findet sich eine grosse Menge
von kokkenförmigen Mikroorganismen, die in manche Klümpchen,
etwas grösser als die Drüsenzelle, eingeteilt sind und an Drüsenzellen

zusammenkleben. Behandelt man dünne Schnitte mit Hämatoxylin-Eosin
und betrachtet man diese unter der starken Vergrösserung, so erkennt
man, dass jedes Klümpchen von Bakterien mit einem an freien Ober-
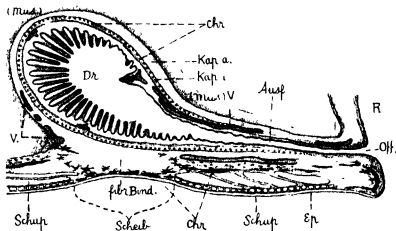flächen der Drüsenzellen angeklebten Säckchen eingehüllt ist. Die

Fig. 1. Langsschnitt durch die Leuchtorgan von *Physiculus japonicus* (etwas
schematisch) *Ausf* Ausführungsgang; *Dr* Drüse, *Ep.* Epithel, *fibr Bind* fibril-
lares Bindegewebe zwischen der Drüse und dem Hautepithel, *Kap a* äussere
Schicht der Kapsel, *Kap i* innere Schicht der Kapsel, *Mus.* Muskelfleisch der
Bauchwand. *Öff* Öffnung des Ausführungsganges, *R* Rektum; *Scheib* schuppen-
freie, schwarze Scheibe am Bauch; *Schup* Schuppen; *v.* Gefäss (Vergr ×24)

Wand des Säckchens ist sehr dünn und färbt sich bei Eosin rot.
Ob es gleich bei der gebrauchteten Färbungsmethode ausser Bakterien
keine Substanz nachweisbar ist, wäre es doch nicht unwahrscheinlich,
dass darin irgend eine Substanz 'zur Ernährung der Bakterien vor-
handen sein sollte.

   Die Säckchen sind im grössten Teil des Schläuches vollständlich,
während in der Nähe der Mündung so zerrissen sind, dass Klümpchen
der Bakterien im Drüsenraum frei getrennt sind' Wenn wir auch
keinen zuverlässigen Grund haben, wodurch wir die Ursache des
Entstehens von Säckchens erklären können, so können wir doch soweit
sagen, dass es sich weder um eine direkte Produktion der Bakterien
selbst, noch um eine Abänderung der Drüsenzellen handelt. Und

wir könnten mit einiger Sicherheit glauben, dass die Säckchen nichts
anders als die durch den Reiz der Bakterien auf der Oberfläche der
Drüsenzellen gebildeten Vorsprünge wären. Es sind natürlich die
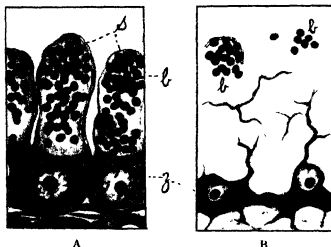festen Grenzmembrane, die den Eintritt der Bakterien in die Zellen

Fig. 2 Säckchen und Bakterien in der Schläuche   Bei A sind
Bakterien von Säckchen vollständig eingehüllt. Bei B sind die Säckchen
zerrissen. b Bakterien; s Säckchen; z Drüsenzellen. (Vergr. ×2000.)

verhindern, zwischen Zellen und Vorsprüngen gebildet. Ja, tatsächlich
sind bei der exakten Betrachtung der Schnittenpräparate keine von
Bakterien befallenden Drüsenzellen zu finden

Alle Schläuchen münden in einen Lumen, der im vorder-oberen
Teil der Drüse liegt, und von dessen Vorderende der Ausführungsgang
läuft; dieser verläuft geradehin durch das Muskelgewebe der Bauch-
wand und mündet endlich im Rektum, nahe an dem After. Das
Epithel und überhaupt die Schleimhaut des Ausführungsgangs zeigen
im Querschnitt starke Längsfalten, noch dazu finden sich in der Nähe
vom Drüsenlumen einige parallel laufende schmale Schläuchen getrennt
ist. Mit jeder Annäherung zu der Öffnung vermindelt sich der Aus-
führungsgang allmählich an Zahl der Scheibenwand, so dass sie end-
lich gänzliche unbemerkbar wird.

In der Kapsel der Drüse sind zwei verschiedene Schichten, die
innere und die äussere, voneinander zu unterscheiden. Die Erstere

besteht aus wellig gekrümmten feinen Fibrillären, die einander fast parallel geordnet sind und sich mit Hämatoxylin-Eosin rot färben; in dieser Schicht finden sich zahlreiche Chromatophoren. Die Letztere dagegen besteht aus einer Durchflechtung von etwas dickeren Fibrillären, die nach verschiedenen Richtungen des Raumes raufen, d. h. aus den kollagenen Fibrillenbündeln. Diese Fibrillenbündeln färben sich mit Hämatoxylin-Eosin bräunlich-rot.

Zwischen der Unterseite der Drüse und der Haut liegt ein Bindegewebe, das aus im Holizon parallel geordneten, feinen Fibrillären besteht. Hier ist das Korium dünner und es fehlt an Schuppen. Die Bauchscheibe ist nur die Aussenseite des Bindegewebes.

Bei lebenden Fischen ist das Bindegewebe etwas durchsichtig und scheint wie die Linse zu wirken, aber tatsächlich ist es nich der Fall, weil Chromatophoren nicht nur im Gewebe, sondern auch zwischen dem Gewebe und der Druse enthalten sind, und zwar es keine Vorrichtung gibt, womit Chromatophoren versetzt werden sollen. Ja, in der Tat sahe ich bis jetzt niemals die Drüse sichtbar in der Natur nach aussen ausstrahlen, es gelang mir nicht auch alle Versuche, im Aquarium von Organen der Fische sowohl durch mechanische als auch durch chemische Reizung Licht ausstössen zu lassen. Demzufolge kam ich zu der Ansicht, dass dieses Organ wahrscheinlich durch den Ausführungsgang hinaus eine Masse von den Leuchtbakterien ins Rektum hinausführe und durch den After in Seewasser ausspücke, um eine leuchtende Wolke zu bilden. Dies ist aber die Meinung experimentell gar nicht bewiesen. Folglich müsste ich auf den Vorwurf erwarten, dass es voreilig wäre, die Drüse als Leuchtorgan zu bezeichnen. Aber die Tatsache, dass die Drüse in sich symbiontische Leuchtbakterien enthält, würde sicher vom genug triftigen Grund sein, um diesem Vorwurf entgegenstehen zu können.

IV. BEOBACHTUNG UND EXPERIMENT DES INHALTES DER DRÜSE

Schneidet man mit dem Messer eine frische Drüse in zwei Teile ab, und sie im Dunkel zu beobachten, so sieht man die Schnittenfläche prächtig grünlich-blau beleuchten. Wenn man fest ein Stück der geschnittenen Drüse mit einem Pinzett fasst, so drückt sich gepresst eine geringe Menge des leuchtenden Schlammes von der

Schnittenfläche aus, wovon seine Emulsion und seine Hängetropfen-
präparate hergestellt werden. Den Rest fasst man, die Schnittenfläche
nach unten, mit einem Pinzett und drückt ihm zur Herstellung der
Tupfpräparate leicht auf eine Objektträger aus.

1) Beobachtung des Schlammes im Tupf- und Hängetropfen-
präparate.

Das Tupfpräparat wird mit dem ZIEL'schen Fuchsin oder dem
Gentianaviolett gefärbt. Es sehen so zahlreiche Bakterien aus, als ob
sie eine Reinkultur der Kokken wären; übrigens sind sie mit wenigen
zelligen Flemmen gemischt.

Hängetropfenpräparat wird von dem Schlamm mit Seewasser oder
3 proz. Kochsalzlösung hergestellt. Die Kokken zeigen keine Eigen-
bewegung. Im Dunkelzimmer kann man das Leuchten der Kokken
unter dem Mikroskop wahrnehmen.

2) Experiment der Emulsion des Schlammes.

Etwa 2-platiose Menge von Schlamm wird mit Platinnadel auf der
inneren Wand des Probeshöhrchens fein zerreibt, 10 ccm 3 proz. Koch-
salzlösung wird hierauf gegossen und das Röhrchen wird kräftig ge-
schüttelt; so wird eine Emulsion des Schlammes gewonnen. Anfangs
leuchtet diese ganze Emulsion, aber später leuchtet nur die Schicht,
welche mit Luft berührt. Aber durch Erschütterung kommt der
frühere Zustand wieder zurück Diese Erscheinung bedeutet, dass
zum Leuchten der Emulsion die grössere Menge von Sauerstoffe not-
wendig ist.

Die leuchtende frische Emulsion wird 30 Minuten (3000mal per
Minute) zentrifugiert; so versinken die Bakterien und die zelligen
Flemmente in Boden des Glasröhrchens vollständig. In diesem Zu-
stand ist keine Licht zu finden. Die klare Flüssigkeit davon wird
hinauf in anderes Röhrchen sorgfältig gegossen und geschüttelt Aber
diese Flüssigkeit bringt kein Licht. Der Bodensatz im ersten Röhrchen
dagegen erschüttert ein starkes Licht aussströmen. Aus diesem Ex-
periment begreift man, dass ausser den Bakterien gar keine Leucht-
substanz, wie sie im Wasser löslich ist, im Schlamm vorhanden ist.

Wenn man diesmal statt der 3 proz. Kochsalzlösung das destillierte
Wasser gebraucht, verliert die Emulsion auf der Stelle das Licht.
Dann um die Emulsion 3 proz. Kochsalz enthalten zu lassen, wird
eine gleiche Menge von 6 proz. Kochsalzlösung tropfenweise hinzu-

gefügt, aber sie findet sich wieder durchaus nicht schimmernd; d. h.
sie hat das Leuchtvermögen auf immer verloren.

Darauf wird nun umgekehrt behandelt; d. h. dem Emulsion mit
3 proz. Kochsalzlösung wird destilliertes Wasser tropfenweise gegossen.
Je kleiner die Salzkonzentration im Medium wird, je schwächer wird
das Leuchten, und wenn die Menge der mit destillierten Wasser ge-
mischte Emulsion 3–4mal so viel als früher beträgt, so kommt endlich
das Leuchten ganz zu Ende. Die Erscheinung ist aber hier nicht
eine solche, wie sie ewige Verlust des Leuchtvermögens bedeutet, denn
das Leuchten wird dann zum Vorschein komme, wenn sich durch die
Hinzusetzung von Kochsalz die Salzkonzentration im Medium vermehrt.

Diese Verhältnisse von Sauerstoff und Kochsalz zum Leuchten des
Schlammes würden beweisen, dass der leuchtende Schlamm eine Masse
von leuchtenden Mikroorganismen sein sollte; worauf es aber beruht,
sei später erwähnt.

V ZÜCHTUNGSPROBE DER LEUCHTENDEN MIKROORGANISMEN.

1) Die Züchtung aus dem Drüse.

Schon durch oben erwähnte Beobachtungen und Experimente wird
es fast bestätigt werden, dass der Inhalt der Drüse aus kokkenartigen
Bakterien besteht die das Leuchtvermögen haben, aber um es zum
entscheidenden Beweis zu bringen, werden die Bakterien aus der
Drüse hinaus auf künstlichen Nährböden zu züchten versucht. Die
Züchtung wird durch folgende Methode ausgeführt: die Drüse wird
mit sterilisierten Instrumenten herausgenommen, wiederholt in 3 Proz.
Kochsalzlösung gespült und mit dem sterilisierten Messer geöffnet;
von dem Inhalt wird mit der Platinnadel auf Nährböden abgeimpft.
Als Nährböden gebraucht man HATTORI's Nährboden für die Leucht-
bakterien und Fleischbouillonagar' und -Gelatine mit 3 proz. Kochsalz
verwendet. Die Reaktion der Nährböden wird mit Zusatz von 1/5
N.-Lösung der Kalilauge auf pH = 7.0 eingestellt.

Bei 30 Individuen der Fische, je 2 Züchtungen aus einer Drüse,
werden bei Zimmertemperatur nach 24 Stunden 60 Stämme der starke
leuchtenden Kulturen gewonnen. Alle Stämme sind schon hier ganz
rein, und bei strengen Prüfung werden sie bestätigt, dass sie zu der-
selben Art der Leuchtbakterie gehören, und zwar dass diese Art die
recht neue ist.

2) Die Züchtung aus dem Darmgang.

Weil der Ausführungsgang der Drüse im Rektum mündet, so würden die symbiontischen Leuchtbakterien ins Rektum geführt werden um endlich durch den After nach Aussen ausgeschieden zu werden. Hier entsteht eine Frage, ob zuweilen ein Teil der ins Rektum geführten Bakterien hinauf im Darmgang gediehen. Um diese Frage zu lösen, wird die Züchtung der Leuchtbakterien aus dem Darmgang versucht.

Die Bauchwand wird herauspräpariert, und an der neun Stelle wie in Fig. 3 geszeichnet, wird der Darmgang mit dem Garn fest zusammengepresst, um die Bewegung oder den Ausfluss des Inhaltes zu verhindern; dann wird der Darmgang aus der Bauchhöhle herausgenommen und in eine sterilisierte Schale verlegt, wo wiederholt mit der 3 proz. Kochsalzlösung gespült; darauf wird die Wand mit einem sterilisierten Messer geöffnet; vom Inhalt wird 1/4 der Plantinöse herausgenommen und in gelöste Nährgelatine gemischt, in die PETRIsche Schale gegossen und bei Zimmertemperatur erhalten.
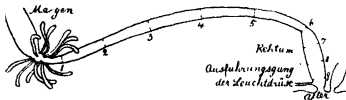
Fig. 3   In der Figur sind die bei der Züchtungsprobe der Bakterien aus dem Darmgang mit dem Garn zusammengepressten Stelle (1-9) zu sehen.

Nach 36 Stunden fangen die leuchtenden Kolonien bei blossen Augen sichtbar zu werden an. Tabelle 1 zeigt die Zahl der leuchtenden Kolonien nach zwei Tagen.

Durch später angestellten ist es gewusst, dass die Gelatine verflüssigenden Kolonien zu einer banalen leuchtenden Wasservibrione gehören, während die Gelatine nicht verflüssigenden Kolonien zu dem symbiontischen leuchtenden Micrococcus gehören.

Der Erfolg der Untersuchung lehrt, dass sich die symbiontischen Leuchtbakterien im Leuchtorgan auch im Rektum befinden. Dieselben sind aber sehr geringer Zahl und um in der begrenzten Gegend der

TAB. I.   Züchtungsversuch der Leuchtbakterien aus
dem Darmgang.

	Zahl der leuchtenden Kolonien aus dem Darmgang.								
	1–2	2–3	3–4	4–5	5–6	6–7	7–8	8–9	9–
Versuch 1	—	—	—	—	—	—	—	**3** / 63	**12** / 59
Versuch 2	—	·	—	··	—	—	57	**3** / 75	**11** / 103
Versuch 3	—	—	—	—	— ·	—	23	**5** / 12	**29** / 11
Versuch 4	—	—	—	—	—	—	17	30	**3** / 70

In der obigen Tabelle zeigen die dicken Schriften die Zahl der die Gelatine
nicht verflüssigenden Kolonien, und die feinen Schriften dieselbe der die Gelatine
verflüssigenden Kolonien

Mündung des Ausführungsgangs vorhanden, daraus folgt, dass diese
Bakterien nicht regelmässig im Rektum gediehen sein.
Ich habe zwar leider nichts darüber zu entscheiden, ob die zugleich
gezüchteten Leuchtbakterien im Rektum der Fische zu wohnen pflegen
oder sich zufällig im Rektum der vier zum Experiment gebrauchteten
Fische sehen liessen. Aber weil men dieselben Vibrionen entweder
aus der Oberfläche der Leiche der Fische oder aus Meerwasser ge-
züchtet sehen kann, sind sie Vibrionen eigentlich als eine Art der
saprophytischen, banalen, leuchtenden Wasserbakterien zu gelten.

VI   MOLPHOLOGISCHE UND KULTURELLE VERHALTEN
DER GEWONNENEN LEUCHTBAKTERIEN

1) Symbiontische Leuchtbakterien (*Micrococcus Physiculus n. sp.*).
60 Stämme der symbiontischen Leuchtbakterien, die je zwei aus
30 verschiedenen Leuchtorganen gezüchtet, zeigen dieselben morpholo-
gischen und kulturellen Verhalten. Auf Bouillonagar oder Bouillon-
gelatine die Stämme senden alle ein so intensives, grünlich-braues
Licht aus, dass man es auch bei Tage im dunklen Schatten leuchten
sehen kann. Wenn die Bakterien bei jeder vermehrten Generation
auf künstlichen Nährboden gezüchtet, zeigen sie, obgleich in Leucht-
organen kokkenförmig gestaltet, eine Neigung, zu kleiner kurze Stäb-
chen zu ändern, z. B. erste Kulturen ist der grösste Teil der Individuen

kokkenförmig gestaltet, aber in Kulturen 15ter Generation (eine Generation dauert eine Woche) sind aber Stäbchen schon beherrschend. Diese Umgestaltung der Individuen jedoch hat keine Veränderung der anderen morphologischen und kulturellen Verhalten im Gefolge.

Die Kokken haben das Durchmesser von 1.0 bis 1.5 μ; die Stäbchen haben die Länge von 1.0–1.5 μ und die Breit von 0.7–1.0 μ. Die Bakterien zeigen keine Eigenbewegung; bei Geisselfarbung nach LÖFFLER und ZETTNOW kamen keine Geissel hervor. Die Bakterien färben sich mit gewöhnlichen Anilinfarbe gut, aber nicht bei GRAMMscher Färbung. Bei Karbolfuchsin treten Vakuolen auf.

Gelatine wird nicht verflüssigt. Auf Gelatineplatten bilden die Bakterien die Kolonien, die anfänglich von der ganz runden, glatten Form, später unregelmässig gelappt und gelblichweiss sind, und um die Kolonien lassen sich bisweilen eine gehäufte Ablagerung von Kristallen bemerken Oberflächen- und Tiefkolonien unterscheiden sich nur an der Grösse voneinander. Die aufliegenden Kolonien auf der Agarplatte sind rund, scheibenartig und ganzrandig, bei auffallendem Licht scheinen sie weiss, aber bei durchfallendem Licht am Zentrum gelblich und am Rand weisslich aus ; die tiefliegenden Kolonien sind dagegen viel kleiner, spindelförmig und gelblich.

Im Bouillon bilden alle Stämme keine Häutchen ; anfänglich werden die Bouillon gleichmässig getrübt, aber später allmählich klarer ; Bodensatz mässig, weisslich, Kohärenz gering ; Indol ist nicht gebildet.

Dextrose, Mannose, Galactose, Maltose und Lävulose werden mit Säure- und Gasbildung von allen Stämmen vergoren, Lactose und Saccharose aber nicht.

<div align="center">

TAB. II.   Zuckervergärung der *Micrococcus*
Physiculus-Stämme.

</div>

Stamm	Dextrose	Lactose	Mannose	Galactose	Maltose	Lävulose	Saccharose
Micrococ. Phys. 1.	rot, Gas	violett	rot, Gas	rot, Gas	rot, Gas	rot, Gas	violett
„ „ 2.	„ „	„ „	„	„ „	„ „	„ „	„
„ „ 3.	„ „	„ „	„ „ ,	„ „	„ „	„ „	„
„ „ 6.	„ „	„ „	„	„ „	„ „	„ „	„
„ „ 7.	„ „	„ „	„	„ ·	„ „	„ ·	„

Zur Prüfung der Zuckervergärung wird gewöhnliche Fleischbouillon,
mit 3 proz. Kochsalz und mit der einzigen Zuckerart gemischt, und
mit Lachmuslösung gefärbt. Die Kulturen werden bei 15°-18°C. ge-
halten, und täglich in einer Woche beobachtet.

In Bezug auf die Alkalität der Nährboden hat der Micrococcus
eine ziemlich grosse Wachstumsbreite. Bei pH=50 fangen erst alle
geprüften Stämme zu wachsen und zu leuchten an. Bei pH=6.5-8.5
gedeihen sie am grossten und leuchten am stärksten; bei pH=9.5
kann man noch, obgleich immer weniger, das Wachstum und das
Leuchten sehen. Ein Beispiel ist im Tabelle III gezeichnet.

TAB. III. Alkalität, Wachstum und Leuchtvermögen bei
Micrococcus physiculus.

	pH=4.5	pH=5.0	pH=5.5	pH=6.0	pH=6.5	pH=7.0	pH=7.5	pH=8.0	pH=8.5	pH=9.0	pH=9.5
Wachstum	−	+	+	++	++	+++	+++	+++	++	++	+
Leuchten	−	+	+	++	+++	+++	+++	+++	+++	++	+

Das Optimumtemperatur für Wachsen und Leuchten bleibt bei
15°-18°C. Die Micrococcus halten gern im niederen Temperatur, wie
z. B. in 3°C., aus, aber im höheren Temperatur zeigen sie eine scharfe
Empfindlichkeit wie im Tabelle IV gezeichnet. Bei 30° erleiden die
Bakterien keine Schadigung an den Wachstumsfähigkeit oder am
Leuchtvermögen, bei 33° aber verlieren sie in 8 Stunden das Leucht-
vermögen, sterben in 24 Stunden, und durch 1/2 stündiges Erhitzen
in 45° kommen sie zu Grunde.

TAB. IV. Temperaturempfindlichkeit des *Micrococcus Physiculus.*

Temperatur	30°		33°				37°		45°
Zeit	12 Std	24 Std.	5 Std	8 Std.	12 Std	24 Std	2½ Std.	5 Std.	½ Std.
Wachstum	+	+	+	+	+	−	+	−	−
Leuchten	+	+	+	−	−	−	−	−	−

Die Temperaturempfindlichkeit wird nach folgender Methode gemessen: Man bringt die 1-tägige Agarkultur in Bouillon, und die Aufschwemmung wird einzeln in verschiedener, bestimmen Zeit bei verschiedener Temperatur gehalten; je 1 Tropfen davon wird Oberfläche einer Agarplatte gebracht und bei 15°-18°C. gehalten um nach 24 Stunden Wachstum und Leuchtvermögen geprüft zu werden.

Differentialdiagnose: Unter bisher bekannten Leuchtbakterien stimmt dieser Micrococcus in mancher Beziehung mit *Micrococcus Sepiola* KISHITANI am meisten überein. Der neu Micrococcus unterscheidet sich jedoch von *Micrococcus Sepiola* 1) durch die Vergärung der Mannose, 2) durch das Fehlen der Neigung von Fadenbildung auf Agar und 3) durch das Vorhandensein des Feuchtglanzes auf dem Belag oder auf dem Auflage.

Nächst dem *Micrococcus Sepiola* weist *Coccobacillus Pierantonii* MEISSNER eine gewisse Änlichkeit mit der neuen Bakterie auf. Aber sie ist mit unipolaren Geissel beweglich und bildet in Zuckervergärung aus Mannit unter blaufärbung Alkali. Unter den banalen Leuchtbakterien können wir keine solchen finden, wie sie nähere Affinität mit dem neuen Micrococcus als zwei oben erwähnte Arten aufweisen. Dies ist der Grund, dass der Micrococcus als eine neue Art angenommen werden soll, die wir „*Micrococcus Physiculus*" n. sp. nennen wollen.

2) Banale leuchtende Wasserbakterien (*Microcpira Asamushiensis* n. sp.).

Alle Stämme der Vibrione, die aus dem Darmgang sowie aus der Hautoberfläche des *Physiculus japonicus* und aus dem Seewasser gezüchtet, zeigen dieselben morphologischen und kulturellen Verhalten. Die Individuen haben die Länge von 1.0-1.5 μ und die Breite von 0.5-0.7 μ. Die Microspira ist mit dem 1-unipolaren Geissel bewglich, und färbt sich mit gewöhnlichen Anilinfarbstoffen gut, aber nach GRAMM nicht. Sie bildet weder Spore noch Kapsel.

Gelatine wird verflüssigt. Anfänglich bildet die Microspira auf Gelatineplatte kleine hohlrunde Kolonie, die später allmählich grösser wird und schliesslich durch die vollständige Auflösung der Gelatine herabgleitet. Auf Agarplatten bildet aber sie auch konkave, dünne Kolonie, die bei durchfallendem Licht gelblichweiss, aber bei auffallendem fast farbloser scheint. Bei Stichkultur wird die Gelatine

glockenförmig verflüssigt. Die Auflage auf der Agarstichkultur ist
scheibenartig, dünn und weisslich. Im Stichkanal ist der Wachstum
gering. Auf der Agarschläge ist der Belag mit dem feinen wellen-
rand etwas ausgebreitet, weisslich und feuchtglänzend.

Bouillon ist gleichmässig getrubt, und in derselben bilden sich
weder Indol noch Häutchen. Milch ist nicht koaguliert, Stärke nicht
hydrolisiert. Nitrat geht in Nitrit über.

Wie in der Tabelle V gezeichnet werden Dextrose, Mannose,
Galactose, Maltose und Lävulose unter Säurebildung vorgoren, aber
es kommt niemals zur Gasbildung, Lactose und Saccharose jedoch
unverändert.

TAB. V.   Zuckervergärung der *Microspira Asamushiensis*-Stämme.

Stamm		Dextrose	Lactose	Mannose	Galactose	Maltose	Lävulose	Saccharose
Microspira Asam.	1	rot	violett	rot	rot	rot	rot	violett
,,	,, 2	,,	,,	,,	,,	,,	,,	,,
,,	,, 3	,,	,,	,,	,,	,,	,,	,,
,,	,, 21	,,	,,	,,	,,	,,	,,	,,
,,	,, 22	,,	,,	,,	,,	,,	,,	,,

In Bezug auf die Wasserstoffionenkonzentration des Nährbodens
zeigt die Microspira eine ähnliches Verhalten mit dem *Micrococcus
Physiculus*. Unter pH = 4.5 bemerkt man keinen Wachstum. Bei
pH = 5.0 fängt sie zu wachsen an, aber sie leuchtet noch nicht. Die
günstigste Kanzentration für Leuchten und Wachsen ist pH = 7.0–8.5
zu sehen, und bei pH = 9.5 sieht man sowohl die Wachstumsfähigkeit
als auch das Leuchtvermögen sehr geschwächt.

TAB. VI.   Alkalität, Wachstum und Leuchtvermogen bei
Microspira Asamushiensis.

	pH = 4.5	pH = 5.0	pH = 5.5	pH = 6.0	pH = 6.5	pH = 7.0	pH = 7.5	pH = 8.0	pH = 8.5	pH = 9.0	pH = 9.5
Wachstum	−	+	+	++	++	++	+++	+++	+++	++	+
Leuchten	−	−	+	+	+	++	++	++	++	+	+

Die Optimumtemperatur für Wachstum und Leuchten fällt auf
15°-18°C. Die Empfindlichkeit der Microspira für die höhere Tem-
peratur ist, wie man in der Tabelle VII sieht, ganz dieselbe wie bei
Micrococcus Physiculus.

TAB. VII.   Temperaturempfindlichkeit der *Microspira
Asamushiensis*.

Temperatur	30°		33°				37°		45°
Zeit	12 Std.	24 Std	5 Std	8 Std	12 Std	24 Std.	2½ Std	5 Std.	¼ Std
Wachstum	+	+	+	+	+	—	+	—	—
Leuchten	+	+	+	—	—	—	—	—	—

Differentialdiagnose: Unter allen bekannten Leuchtbakterien stehen
Microspira delgadensis (FISCHER) MIG. und *Microspira luminosa*
(FISCHER) MIG. in naher Beziehung mit meiner Microspira. Die
Beschreibungen, die ersteren betreffend, sind aber so kurze und ein-
fach, dass man die neue Microspira nicht genau mit diesen zwei Arten
der Bakterien vergleichen kann. Aber die Erstere ist von *Microspira
luminosa* wenigstens dadurch, dass sie nicht von der Neigung ist,
sich zu längeren Spirillen zu verbinden und sich zu Bakteroiden zu
bilden, und von *Microspira delgadensis* durch ihre Kolonienform zu
unterscheiden.

Unter den neuere Zeit befundenen Leuchtbakterien stimmt *Vibrio
sulla Sepia* MEISSNER in vielen Punkten mit meiner Vibrione überein,
aber die erstere verflüssigt Gelatine nicht und bildet Häutchen in
Bouillon, was aber bei der letzteren nicht der Fall ist. Diese beiden
Bakterien unterscheiden sich von einander auch durch das Verhalten
in der Zuckergärung und in der Alkalität des Nährbodens. Ich nehme
daher die Microspira als die neue Leuchtbakterien an will demzufolge
dem Namen der Ort, wo sie zum erstenmal gefunden wurde, „ *Micro-
spira Asamushiensis* " n. sp. nennen.

VII. AGGLUTINATORISCHE VERHALTEN DER
GEWONNENEN LEUCHTBAKTERIEN.

Dass die mit den symbiontischen Leuchtbakterien, wie z. B. *Cocco-bacillus Pierantoni, Vibrio Pierantonii, Pseudomonas Euprymna, Micrococcus Sepiola* und *Coccobacillus Loligo,* hergestellten Kaninchenimmunseren eine ausgesprochene Stammesspezifität im agglutinatorischen Verhalten aufweisen, und dass bei den banalen leuchtenden Wasserbakterien, wie z. B. *Coccobacillus sulla Sepia, Vibrio sulla Sepia, Pseudomonas luminescens, Pseudomonas photogena, Pseudomonas phosphorescens* u. s. w. dagegen keine Stammesspezifität besteht, sind schon von MEISSNER und von mir ins klare gebracht. Um zu sehen ob bei der neu gewonnenen leuchtenden Micrococcus und Microspira die obigen Verhalten entstehen, werden folgende Experimente angestellt.

Durch intravenöse Injektion werden 6 Kaninchen dreimal alle sieven Tage mit dem 3 verschiedenen *Micrococcus Physiculus*-Stämme und 3 verschiedenen *Microspira Asamushiensis*-Stämme immunisiert (die Dosis steigt jedesmal zweimal, 0.5 bis 2.0 mg). Eine Woche nach der letzteren Injektion wird das Blut total entgenommen, von dem werden Immunseren hergestellt. Bei 10 *Micrococcus Physiculus*-Stämmen und 10 *Microspira Asamushiensis*-Stämmen wird die Agglutination jedes der Immunseren, die mit 0.8 proz. NaCl-Lösung in der Weise wie 1 : 50, 1 : 100, 1 : 200 u. s. w. verdünnt sind, geprüft.

Die Ergebnisse der Versuche bieten, wie in Tabellen VIII und IX gezeichnet, nicht Besonders, d. h. bei den symbiontischen Leuchtbakterien, dem *Micrococcus Physiculus* werden nur der Ausgangsstamm und der von demselben Fischindividuum herkommende Stamm mit dem sehr verdünnten Serum agglutiniert, während die übrigen Stämme, die zu derselben Art gehöhren, nur bei ganz hochwertigen Seren (bis zur 1 : 50) oder gar nicht mitagglutiniert werden ; alle Stämme erweisen aber bei banalen leuchtenden Wasserbakterien, der *Microspira Asamushiensis*, dass sie im agglutinatorischen Verhalten entweder identisch oder mindestens in naher Verwandtschaft sind.

In obigen Tabellen sind als Werte diejenigen Verdünnungen angegeben, bei denen eine im Agglutinoskop gerade noch deutlich agglutination auftrat, aber „O” zeigt, dass bei Verdünnung unter 1 : 50 (z. B. 1 : 25 u. s. w.) keine Agglutination auftrat.

Da *Micrococcus Physiculus*-Stämme wie z. B. 5a und 5b oder 7a

TAB. VIII. Endtiter der Agglutination von *Micrococcus Physiculus*-Immunseren mit *Micrococcus Physiculus*-Stämmen.

	Mic. coc. Phys. 5 a	Mic. coc. Phys. 5 b	Mic. coc. Phys. 7 a	Mic. coc. Phys. 7 b	Mic. coc. Phys. 7 1	Mic. coc. Phys. 10 a	Mic. coc. Phys. 10 b	Mic. coc. Phys. 1 a	Mic. coc. Phys. 2 a	Mic. coc. Phys. 11 a	Mic. coc. Phys. 15 b
Mic. coc. Phys 5 a — Im Ser	3200	3200	0	0	0	0	0	0	0	0	0
Mic. coc. Phys 7 a — Im Ser.	50	50	6400	3200	50	50	0	0	50	0	
Mic. coc. Phys 10 a — Im Ser	0	0	0	0	3200	3200	0	0	0	0	

TAB. IX. Endtiter der Agglutination von *Microspira Asamushiensis*-Immunseren mit *Microspira Asamushiensis*-Stämmen.

	Mic. spir. Asam. 5	Mic. spir. Asam. 7	Mic. spir. Asam. 10	Mic. spir. Asam. 22	Mic. spir. Asam. 1	Mic. spir. Asam. 23	Mic. spir. Asam. 4	Mic. spir. Asam. 6	Mic. spir. Asam. 8	Mic. spir. Asam. 9
Mic. spir Asam. 5 — Im. Ser.	3200	400	400	1600	400	400	200	400	800	3200
Mic. spir. Asam. 7 — Im Ser.	800	6400	50	800	400	400	400	800	800	1600
Mic. spir. Asam. 10 — Im Ser	800	50	6400	800	200	800	200	800	1600	1600

TAB. X. Endtiter der Agglutination von *Micrococcus Physiculus*-Immunseren mit *Microspira Asamushiensis*-Stämmen und von *Microspira Asamushiensis*-Immunseren mit *Micrococcus Physiculus*-Stämmen.

	Mic. coc. Phys. 5 a	Mic. coc. Phys. 7 a	Mic. coc. Phys. 10 a	Mic. spir Asam. 5	Mic. spir. Asam. 7	Mic. spir Asam. 10
Mic. coc. Phys. 5a — Im. Ser.				0	0	0
„ „ 7 a — „				0	0	0
„ „ 10 a — „				0	0	0
Mic. spir. Asam. 5 — Im. Ser.	0	0	0			
„ „ 7 — „	0	0	0			
„ „ 10 — „	0	0	0			

und 7b u. s. w. aus demselben Leuchtorgan entstammt sind, werden
sie bei den entsprechenden Immunseren im derselben oder mindestens
fast nahestehenden Verdünnung mit dem Ausgangsstamm agglutiniert.
Daraus folgt es, dass der *Micrococcus Physiculus* zu betreffenden
Tierindividuen streng angepassend ist.

    Ferner wird die Agglutination zwischen dem *Micrococcus Physiculus*
und der *Microspira Asamushiensis* geprüft, aber sie voneinader, wie
man in der Tab. X sieht, gar nicht.

                              VIII DISKUSSION

    Das Leuchtorgan der *Malacocephalus laevis* (LOWE) mit dem von
Physiculus japonicus HILGENDORF zu vergleichen, ist sehr interesièrt.
Diese beiden Fischarten gehöhren zu derselben Gruppe, zur *Anacantini*
(*Gadiformes*), und dazu zeigen ihre Leuchtorganen in dem Struktur
miteinander eine ausserordentliche Ähnlichkeit. Aber sie unterscheiden
sich von einander nur in der Art der Lichtproduktion.

    Ich will durchaus nicht an Ergebnisse der Untersuchungen HICKLINGS
über das Leuchtorgan von *Malacocephalus laevis*, zweifeln, aber ich
möchte hier eine Angelegenheit haben, die meine Ansicht darüber zu
äussern.

    Unter den Beschaffenheit des Sekretes, die durch HICKLINGS
Experimente in klare gebracht worden sind, hat besonders meine
Aufmerksamkeit erregt, 1) dass für dass Leuchten des Sekretes eine
ziemlich grosse Menge des Sauerstoffes notwendig ist ; 2) dass wenn
das Sekret ins destilliertes Wasser direkt hingeworfen, das Leuchten
plötzlich aufgibt, und sogar das Leuchtvermögen, wenn man auch das
mit dem Seewasser behandelt, nicht mehr zurückkommt ; aber wenn
das Sekret zuerst mit einer kleinen Menge von Seewasser behandelt
wird, und dann mit einer grossen Menge von destilliertem Wasser
verdünnt wird, kein solches plötzliches Erloschen der Leuchte stattfindet ;
3) dass in solchem mit destilliertem Wasser verdünnten Medium die
Leuchte des Sekretes früher erlescht als in Seewasser selbst. Diese
obigen Beschaffenheit sind es, die mit dem gewöhnlichen Begriff
der bis jetzt bekannten tierischen Leuchtsubstanzen, Luciferin und
Luciferase, sehr schwierig, aber dadurch, dass man dieses Sekret als
Bakterien voraussetzt, sehr leicht zu erklären sind.

Es ist eine wohl bekannte Tatsache, dass die Bakterien beim Leuchten eine weit grössere Menge des Sauerstoffes als Leuchtsekret (Luciferin) konsumiert. In der Tat gab Harvey durch diese Tatsache an, dass die Bakterien die Lichtquelle der Leuchtorganen von *Anomalops* und *Photoblephalon* sind, was er durch seine bakteriologischen Versuche nicht bestätige konnte. Es ist auch von allen bekannt, dess die Bakterien, mit Ausnahme von einigen im Süsswasser lebenden Arten, wie *Microspira phosphoreum* YASAKI, für den Leuchten eine gewisse Menge von Kochsalz im Medium enthalten müssen. Nach meinen Untersuchungen ist die für das Leuchten geeigneteste Konzentration des Kochsalzes ist von 2 bis 4 Proz., und die für das Wachsen günstigste Konzentration desselben von 2 bis 3 Proz., aber die meisten Leuchtbakterien können auch beim 1.0 Proz. leuchten und beim 0.5 Proz. wachsen. Natürlich wird bei solcher verdünnten Kochsalzlösung das Leuchtvermögen im hohem Grade gehemmt, Beleuchtung kürzer. Verhält es sich das Leuchtsekret vom Leuchtorgan der *Malacocephalus laevis* zu Kochsalz in der Medium nicht so sehr ähnlich wie die Leuchtbakterien? Nimmt man das Sekret als Leuchtsubstanz, wie Luciferin und Luciferase, an, ist die eigentümliche Beschaffenheit schwer zu klaren, dass, wenn man anfangs das Sekret mit Seewasser behandelt, dann mit jeder Hinzusetzung von destilliertem Wasser das Medium zur höhem Verdünnung bringt, das Sekret das Leuchtvermögen nicht verliert, während bei direkt Behandelung mit destilliertem Wasser das Erlöschen der Leuchte stattfindet. Gesetzt, dass das Sekret die Leuchtbakterien sein, kann man es dadurch leicht erklären, dass man diese Erscheinung auf die Veränderung des osmotischen Drückes im Medium zurückführt. Es ist eine klare Tatsache, dass die im verhältnismässig dicken Kochsalzlösung lebenden Microorganismen wenn sie im destillierten Wasser direkt verlegt werden, wegen der plötzlichen und starken Veränderung des osmotischen Drückes in der Umschliessung zu körperlichen Zerstörung gebracht werden, aber wenn diese Änderung langsam ist, wenn auch ziemlich gross, die Microorganismen sich durch ihre Anpassungsfähigkeit von der Gefahr entgehen können. Das Leuchten der Bakterien mag damals ganz oder bis zu einem gewissen Grade gehemmt werden, aber wenn die Umschliessung sich wieder im für Bakterien günstigen Zustand befände, mag das Leuchten auch wieder lebhaft werden.

Sowohl aus dem Bau des Leuchtorganes wie aus dem Verhalten
des leuchtenden Inhaltes scheint die Leuchtsubstanz der *Malacocephalus
laevis* die symbiontischen Leuchtbakterien und nicht das leuchtende
Sekret zu sein. HICKLING aber spricht gegen das Vorhandensein der
Leuchtbakterien, sondern behaupt, dass er im Inhalt eine Leucht-
substanz, Luciferin und Luciferase, geprüft könnte. Wenn es keine
Fehler in seinem Experiment wären, woran ich auch glauben möchte,
können wir über die Grille der Natur nicht genug erstaunen, wie sie
in den Leuchtorganen von *Malacocephalus* und *Physiculus* gezeigt wind.

Hier ist aber zu berücksichtigen, dass *Malacocephalus* einer der
Tiefseebewohner ist, während *Physiculus* einer der verhältnismässig
oberflächlicher ist. Soweit man jetzt weiss, gibt es keine solchen
Tiefseebewohner, in deren Leuchtorganen die symbiontischen Bakterien
leben. SKOWLON ist z. B. sogar der Meinung, das die Leuchtsymbiose
sich nur bei den oberflächlichen Tieren bemerken könnten. Seiner
Ansicht nach, die natürlich auf einiger Sicherheit gegründet ist, könnte
es vielmehr als richtig betracht werden, dass das Leuchten der *Malaco-
cephalus* auf keine Bakterien beruht. Dennoch ist es doch sehr zu
bewundern, dass sich das Leuchtsekret der *Malacocephalus* sehr ähnlich
wie das der Leuchtbakterien verhält.

IX   ZUSAMMENFASSUNG

1. Es ist die im Muskelfleisch der Bauchwand der *Physiculus
japonicus* HILGENDORF befindliche Drüse, die einst dem FRANZ ein
Rätsel war, jetzt endlich dadurch zu erklären gelungen, dass sie ein
Leuchtorgan ist, wo symbiontische Leuchtbakterien leben.

2. Die Drüse ist von einer bindegewebigen Kapsel umgeben, die
in sich eine Schicht von Chromatophoren enthält, und es ist unter
derselben ein Bindegewebe, das aus in holizontalen Richtung parallel
gelegenen Fibrillären hergestellt ist und das sich auf der Oberfläche
des Bauches als eine kleine, schuppenfreie, schwarze Scheibe sehen
lässt.

Nach der Bildung will ich die Drüse als ein Leuchtorgan, das
durch den Ausführungsgang ein leuchtendes Material, Leuchtbakterien,
nach Aussen ausspückt.

3. Anfänglich sind in der Drüsenschläuche die Massen der Bak-

terien in manche, kleine Klümpchen eingeteilt, die in dünnen Zellori-
ginalen Membranen eingehüllt sind; diese mögen vielleicht bei der
Reizung von Bakterien aus Schläuchesepithel gebildet werden, aber
sie werden späterhin zerrissen und so gehen die Bakterien nach dem
Drüsenraum hinaus.

4. Ausser den Leuchtbakterien gibt sich in der Drüse keine
Leuchtsubstanz zu beweisen, deswegen sind die Bakterien als einzige
Lichtquelle des Organs zu gelten.

5. Aus dem Leuchtorgan werden die symbiontischen Leucht-
bakterien auf künstlichen Nährböden gezüchtet. Alle Stämme, aus
den Leuchtorganen von 30 verschiedenen Tierindividuen weisen dieselben
morphologischen und kulturellen Verhalten auf, deshalb müssen sie
als eine und dieselbe Art angenommen werden. Nach einer strengen
Vergleichung mit anderen bisher bekannten Leuchtbakterienarten wird
sie als eine neue Art mit dem Namen von „ *Micrococcus Physiculus* "
n. sp. genannt.

6. Aus der Hautoberfläche und dem Darmgang der *Physiculus
japonicus* wurde manche Stämme von banalen, leuchtenden Wasser-
vibrionen gezüchtet. Die Vibrione gilt als eine neue Art von der
Leuchtbakterien und wird mit dem Namen von „ *Microspira Asamushi-
ensis* " n. sp. genannt.

7. Im agglutinatorischen Verhalten weisen die Stämme der *Micro-
coccus Physiculus* eine ausgesprochene Stammesspezifität auf, aber bei
der *Microspira Asamushiensis* besteht keine solche Stammesspezifität
und die Verschiedenheit dieser zwei Leuchtbakterien im agglutinatori-
schen Verhalten, wie man schon von Untersuchung MEISSNERS und den
meinigen, die alle bei anderen Bakterien ausgeführt würden, sehen
kann, ist ganz von ihnen verschiedenen Lebensgang abhängig.

8. Es ist zu bewunden, dass das Leuchtorgan der *Physiculus
japonicus* und derselbe *Malacocephalus laevis*, wenn auch in Bau sehr
ähnlich, in der Art der Lichtproduktion ganz verschieden sind, d. h.
das erstere in sich als Lichtquelle symbiontische Leuchtbakterien ent-
hält, das letztere aber die Leuchtsekreten, Luciferin und Luciferase,
absondert.

Zum Schluss möchte ich die Ehre haben, meinem geehrten Führer,

Herrn Prof. Dr. H. HATTORI, für die freundliche Anleitung meinen herzlichsten Dank auszusprechen, und ich bin noch verpflichtet dem Direktor des Asamushi Marine-Laboratorium für Biologie, Herrn Prof. Dr. S. HATAI und meinem vertrauten Freunde Herrn D. INABA wegen der grossen Freundlichkeit, die sie bei meinem Studium gegeben hat, grossen Dank zu schenken.

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TAFELERKLÄRUNG.

TAF. XXVII.

Fig   1.   *Physiculus japonicus* HILGENDORF, von rechts gesehen Verger. 1·2.
Fig   2.   Derselbe, zur Demonstration der schuppenfreie, schwarzen Scheibe von
           der Ventralseite gesehen. Vergr. 2:3. A. After; O. Offnung des Uro-
           gemtalkanals, S. Schuppenfreie, schwarze Scheibe.

TAF. XXVIII.

Fig. 3.   Das Leuchtorgan im Längsshnitt. Färbung mit Hämatoxylin-Orange G.
           18mal vergrössert.
Fig. 4.   Dasselbe im Querschnitt. Farbung mit Hämatoxylin-Eosin. 22mal
           vergrössert
Fig. 5.   Querschnitt des Ausführungsgangs. Färbung mit Hämatoxylin-Eosin
           120mal vergrössert.
Fig. 6   Querschnitt der Mündungsteiles des Ausführungsgangs. Fahrbung mit
           Hämatoxylin-Orange G. 120mal vergrössert.

TAF. XXIX.

Fig. 7.   Stück eines Querschnittes durch ein Leuchtorgan 400mal vergössert.
           Im Präparat sind die Drüsenschläuche im Längsschnitt zu sehen Farbung
           mit Hämatoxylin-Eosin
Fig 8.   Dasselbe. 400mal vergrössert Im Präparat sind die Drüsenschläuche
           lin Querschnitt zu sehen. Färbung mit Hämatoxylin-Orange G.
Fig 9   Stück eines Längsschnittes durch ein Leuchtorgan. 1500mal vergrossert
           Das Präparat wurde mit Hämatoxylin-Eosin gefärbt, um besonders die
           die Bakterien einhüllenden Säckchen in den Schläuchen darzustellen.
Fig. 10.   Dasselbe 1500mal vergrössert. Das Präparat wurde mit Hämatoxylin-
           Orange G. gefärbt, um die Gestalt der Bakterien in den Schläuchen klar
           darzustellen.

TAF. XXX.

Fig. 11.   *Micrococcus Physiculus* n. sp. Tupfpräparat des Inhalts der Leuchtdrüses.
           Färbung mit Anilinfuchsin. 1500fach vergrössert
Fig. 12.   Dieselbe. Ausstrichpräparat einer erste 24stünd. Agarkultur 1500fach
           vergrössert. Färbung mit Anilinfuchsin.
Fig. 13.   Dieselbe. Ausstrichpräparat einer 24stünd. Agarkultur 15ter Generation.
           1500fach vergrössert. Färbung mit Anilinfuchsin.
Fig. 14.   Dieselbe. 3tägige Gelatinekolonie. 70fach vergrössert.
Fig. 15.   *Microspira Asamushiensis* n. sp. Ausstrichpräparat einer 24stünd.
           Agarkultur. 1500fach vergrössert. Färbung mit Anilinfuchsin
Fig. 16.   Dieselbe. Geisselfärbung nach LÖFFLER. 1500fach vergrössert.
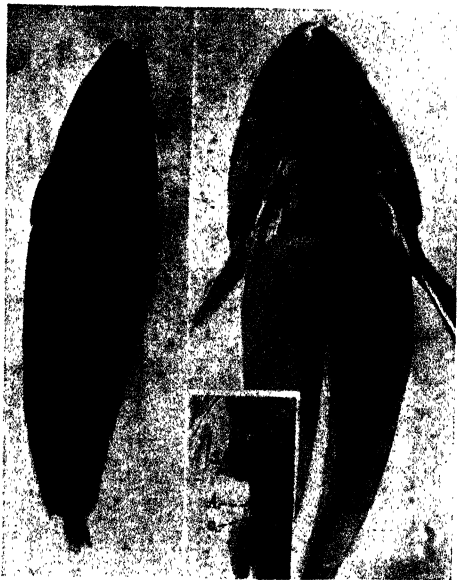
Fig. 1.   Fig. 2

T. KISHITANI: Leuchtsymbiose in *Physiculus japonicus.*

Fig. 3

Fig. 4

Fig. 5.

Fig. 6.

T. Kishitani: Leuchtsymbiose in *Physiculus japonicus*.

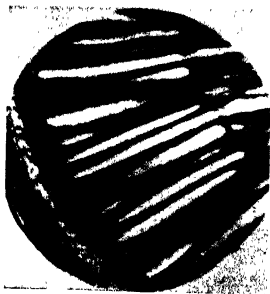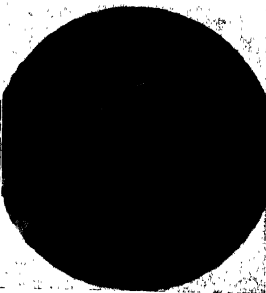Fig 7

Fig. 8

Fig. 9.

Fig. 10.

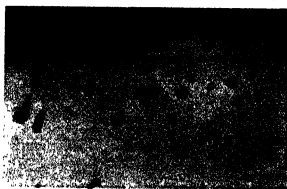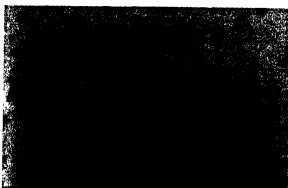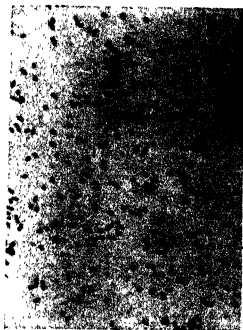T. Kishitani: Leuchtsymbiose in *Physiculus japonicus*.
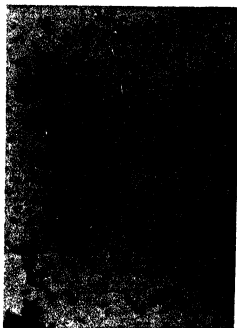
Fig. 11

Fig. 14

Fig. 12

Fig. 15

Fig. 13.

Fig. 16

T. KISHITANI: Leuchtsymbiose in *Physiculus japonicus*.

Comparative Notes on the Japanese Mullets *Mugil cephalus* and *M. haematochilus* *

BY

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(With 2 text-figures).

Among other studies carried out as a result of an Elizabeth Thompson Science Fund grant making it possible for me to travel to and from northern Hondo where I consequently spent most of July and August, is the present comparison of the specific and scale characters of two fish of economic importance, based on my earlier study of the Jumping Mullet (1) My thanks are also due the director and associates for their hospitality and many kindnesses extended during my studies

MATERIAL EXAMINED

At an early season, and in a region of few short rivers and a very rocky coast, but six specimens of *M. haematochilus* were secured These measured 127, 132, 144 154 186, 421 mm in total (greatest) length A single specimen of *M. cephalus* 429 mm long was secured at the same hawl, making excellent comparative material with the 421 mm *M. haematochilus* On returning to Tsinan, two specimens of *M. haematochilus* 555 and 565 mm long were secured from the Tsingtao market August 27th where there were several others as well as two *M. cephalus* October 5th ten *M. cephalus* 285-316 mm long were taken from the Tsingtao market from among a hundred or more There were no *M. haematochilus* during that week

M. cephalus (2) was based on ARTEDI s description of a European species *M. œur* (3) was described from the Red Sea, while *M. japonicus* (4) was characterized and badly figured from Nagasaki specimens Later (5) a Swatow specimen 9 inches long was referred

to *M. oeur*, with *M. japonicus* noted as a synonym, and characterized as head 4; depth 4.5; eye 4; scales 38–13; D. IV–8; A. III, 8. This scale count is within the limits of variation (1, p. 201, lines 3–8). In 1901 (6) Yokohama material was reorded as *M. oeur* with the remark, "We refer the common mullet of Japan (*M. japonicus*) to *M. oeur* of the Red Sea, following the opinion of authors, having no data of our own ". The next year (7) several specimens from Formosa were published as *M. oeur* with scales 40–12 ; D. IV–I, 8 ; A. III, 8. This is nearly typical *M. cephalus*. In 1905 (8) three Hongkong specimens 5.5″–6.5″ long are referred to *M. cephalus* with *M. oeur* as synonym. These juvenile fish are described as : head 3.75 ; depth 4.15 ; eye 4.25 ; D. IV–8 ; A. III, 8 ; scales 39. The same year the same ichthyologists (9) record one Shanghai 5.5″ specimen and one Hongkong 10″ example in the same way but characterize it as : head 4 ; depth 4.3 ; eye 4 ; D. IV–9 [juvenile stage] ; A. III, 8 ; scales 37. Thus the proportions are more like those of (5) ; the dorsal fin formula changed to adult condition would be D. IV–1,8 thus making the fins also *M. cephalus*, although the scale count is low. The next year (11) Formosan specimens appear as *M. oeur* again ! Finally the Japan Catalogue (12) records *M. japonicus* as *M. cephalus*.

A change is then instituted (13) when *M. cephalus* is separated from *M. oeur* on the basis of mandibular angle and a slightly different scale count. The tables of measurements (pp. 270 and 272) leave one very much in doubt as to any specific distinctness, especially if one considers only the three adults of *M. oeur*. In one case, the eye is recorded as 1.77 ! Both species are striped. According to the writer's (1) find of North Carolina *M. cephalus*, the number of scales in longitudinal row varies from 38–44. Even in the Formosan (13) specimens of *M. cephalus* one finds a variation of 40–42, and 38–41 in the nine specimens of *M. oeur* ! The mouth angle may be a factor which is set in death. Thus I cannot consider *M. oeur* and *M. cephalus* as distinct species until much more intensive studies have been made.

Three years later (14) the same writer separates *M. japonicus* from *M. cephalus* on the basis of 38 scales in lateral series and the depth ratio. The latter I find to vary with age and quantity of roe. The Formosan specimen of *M. japonicus* (i. e. the deep " species ")

is 600 mm. long, thus an unusually large specimen. The lateral series is equaled by the Formosan *M. oeur*. On p. 245 one reads, " It is distinct, however, from *M. oeur* in having mandibular bones which meet at an obtuse angle [the "characteristic" of *M. cephalus*] and 38 or 39 scales in lateral series [*the* number for *M. oeur*]! Thus at best *M. japonicus* as described in 1921, should have been regarded as a hybrid of *M. cephalus* and *M. oeur*.

Finally I furnish a table consisting of seven groups of measurements taken from the papers as cited in the left hand column:

	Depth	Head	Eye	Scales
5, 8, 9,	3.75–4.0	4.15–4.5	4.0 –4.25	37–40
13, yn. *M. oeur*	3.42–3.89	4.0 –4.58	3.55–4.66	38–41
13, ad. *M. oeur*	3.91–4.11	4.1 –4.37	1.77–4.0	38–40
13, *M. cephalus*	3.69–3.95	4.09–4.79	4.23–4.85	40–42
14, *M. japonicus*	4.0	4.9	4.47	38
15	4.5	5.0	5.5	38
16	3.0 –4.0	3.5 –4.2	3.2 –4.0	35–40

From this table it is evident that the Hawaiian ratios (16) range lower than the east Asian. Is this due to method of measurement or is it actual ? The Hangchow records (15) range much higher than any others and thus have the appearance of being erroneous. The other groups are not distinctive — especially when one takes size into consideration. It is to be hoped therefore, that the foregoing will once and for all bury these synonyms and forestall further attempts at establishing " new " species on this variable, circum-subtropical, migratory species.

As to the species with restricted adipose eyelid, one must first determine its generic position. The group *Liza* was separated from *Mugil* (17) for species having the eyelid obsolete (which means, not very distinct). Thus it is incorrect to publish "lacking ", as has often been done. This is paramount to saying that even though the type : *M. capito*, an east Atlantic species, should be entirely without adipose tissue, species with the adipose eyelid slightly developed *might* be

included in the genus (Liza was proposed as a subgenus). The ten-
dency, in recent years, has been to transfer more species from Liza to
Mugil. For instance, as pointed out in the Oceania report (16, pp. 124–
126) M. vaigiensis, M. macrolepis, M. seheli, M. crenilabis have been
placed in Liza by several ichthyologists (including JORDAN and) though
now definitely recognized as Mugil. Likewise the present species,
having the adipose eyelid distinctly developed, belongs more truely in
Mugil than in Liza. It is true that there is a considerable degree of
difference between the eyelid in M. cephalus and M. haematochilus
but it should also be remembered: (1) that the eyelid is distinctly
developed and (2) that the eyelid is not developed in the young of
either species but developes as gradually as the fish grows. Thus it
is imperative to consider only the full grown in deciding the generic
status. Authors should always record the length of their specimens.
   Since the single generic character of Liza is one of degree, and
since the accretion of the generic character of Mugil is one acquired
during growth, two facts must be recognized: (1) it is of subgeneric
value at best, (2) lack of adipose eyelid is the more primitive condition.
This latter statement is equivalent to saying that Liza is more primitive
than Mugil and should therefore be placed first in systematic reports,
lists, etc.
   Mugil (Liza) haematochilus is recorded from Hakodate (18), Tokyo
(19), Misaki (20), Fusan: Chinnampo, Chemulpo (21), Antung (22)
L. formosae (14) based on a five inch specimen (young) described with
the dorsal fin formula in the juvenile stage! is covered by the amount
of variation found in specimens of M. haematochilus analyzed for the
present report

              SPECIFIC CHARACTERS (of adults)

M. cephalus	M. haematochilus
Behaviour	
Jump high over net*	Skulk over edge of net
Slow fall migration	Rapid fall migration
Usually dies with mouth closed	Usually dies with mouth open

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   * It is suggested that the term Jumping Mullet be officially restricted to M. cephalus,
   as M. haematochilus is even more heavily stripped than M. cephalus.

Coloration

Stripes less prominent*	Stripes more prominent by reason of enlargement of pigment spot at center of scale pocket rim.
Fins white	All fins except first dorsal with yellowish tinge.

Fin formulae

Anal formula is III, 8	III, 9
2nd D. rays 1 to 2 scaled 2/3 to 3/4 length; ray 3 scaled 1/2 way at most; ray 4 with a few scales at base *if* rays 1 and 2 are scaled 3/4 length	less scaled
Anal scaled as dorsal	as *M. cephalus*

Adipose eyelid

Extends over iris to pupil, forming elongate-oval opening	Extends over outer rim of iris, forming nearly circular opening.
So thick as to conceal scales behind eye in alcoholics.	Scales behind eye clearly visible through the tissue in alcoholics.
Anteriorly extending far anterior to angle of mouth	Anteriorly extending to transverse plane of angle of mouth.
Posteriorly extending a distance equal to distance from anterior nostril to eye center.	Posteriorly extending a distance equal to diameter of eye opening.

Nostrils

Anterior nostril nearer anterior edge of preopercle than to posterior nostril	Anterior nostril nearer posterior nostril than to anterior edge of preopercle

Mouth

Forming an angle of 92°–93°; the ends recurved; closed.	Forming an angle much greater than 90°; sides perfectly straight; open

Ratios

Dorsals more approximate, i. e. length of 1st D. spine nearly	Dorsals more remote, i. e. length of 1st D. spine much less than

equaling distance from base of 1st D. to insertion of 2nd D.	distance from base of 1st D. to insertion of 2nd D.
Insertion of 2nd D. to insertion of caudal equals length of head	Insertion of 2nd D. to insertion of C. much less than length of head
Depth — varies with age in both species.	
Peduncle more slender, i.e. length of *base* of 2nd D. is greater than depth of peduncle	Peduncle deeper, i.e. length of base of 2nd D. shorter than depth of peduncle (equal in yn.)

Skeletal

Epiotic and exoccipital processes (see 23) shorter	Longer
No processes on vertebrae	Similar processes springing from posterior edge of vertebra 2.
Hypural with short spine on side half length of vertebra 23.	Hypural spine slightly longer.

Scalation

Scales on median row	35–44	37–42

Number of longitudinal scale rows from and including lateral row:

to mid-dorsal line to 1st D.	6	5 (+median)
to 1st D.	6 (+accessory)	5 (+accessory)
to between dorsals	7 (no median)	6 (+median)
on peduncle	6	5 (+median)

(That is, there is one longitudinal row less *above* median row)

to before ventrals	11	10 (+median)
to above ventrals	8	7 (+small)
to between V. and A.	9–10 (+median)	8 (+med. at cent.*)
to above anal	6– 7 (+axillary)	5–6 (+axil. *group*)

(That is, there is one longitudinal row less *below* median row, to ventrals. Since there are two longitudinal rows less in *M. haematochilus* and the fish are of the same-relative depth, the scales must be, and are, correspondingly larger).

Number of scale rows from 1st D. to V.   13 rows   11 rows (+small)

Scale 10 of median row (1, fig. 1) becomes scale 8 of median row.

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*Venter often very irregular, with much running together.

(That is, there are two transverse rows less anterior to 1st D.)

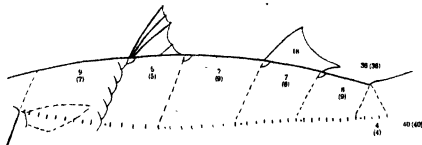First dorsal is inserted on 9th transverse row   7th

Fig. 1. Disposition of numbers of transverse scale rows in *M. cephalus* and *M. haematochilus* (in parentheses)

In order to endeavor to determine why or in what area the number of *transverse* rows (lateral line count) varied as much as it does, the arrangement of these transverse rows in the ten Tsingtao *M. cephalus* were studied. This was done by dividing the area above the lateral line into five areas: predorsal (pre D), subdorsal (sub D), interdorsal (inter D), subdorsal (sub d), and postdorsal (post d), see figure 1. Pins were inserted into the scales of the median row (lateral line row) which belonged to the following transverse rows: (1) that immediately behind the gill cover, (2) that below origin of first dorsal, (3) that originating immediately *behind* posterior edge of first dorsal when the membrane is complete (not broken back) as in figure 1, (4) that originating below origin of second dorsal, (5) that originating immediately *behind* posterior edge of second dorsal, (6) that originating below origin of caudal (see odd scales in figure 1 on mid-dorsal line). The number of scales between these pins were tabulated. The results of five of the fish chosen at random, as well as the two Tsingtao *M. haematochilus* are given in tabular form. It will be seen that there is a great deal of variation but that this variation is chiefly in two areas (1) that below and behind first dorsal, (2) the area flanking the hypural plate (posterior part of caudal peduncle).

Distribution of Transverse Scale Rows

Length		pre D	sub D	inter Dd	sub d	post d	sum	hypural	total
				M. haematochilus					
555 mm.		8	5	9	6	9	37	4	41
565 mm.		7	5	9	7	10	38	4	42
				M. cephalus					
316 mm.	l.	9	5	7	7	8	36	3	39
	r.	9	5	8	7	8	37	4	41
312 mm.	l.	9	6	7	7	7	36	4	40
	r.	9	6	7	7	7	36	5	41
298 mm.	l.	10	7	6	7	9	39	4	43
	r.	9	7	5	7	8	36	1	40
290 mm.	l.	9	5	6	8	8	36	3	39
	r.	9	5	6	7	8	35	4	39
285 mm.	l.	8	6	6	7	8	35	3	38
	r.	8	5	5	7	8	33	3	36

Ratios for these five specimens were: depth 4.33–4.75; head 4.0–4.4; eye 4.25–4.66; snout 3.66–4.0; mouth width 3.25–3.66; inter-orbital 2.25–2.4. The ratios for the two Tsingtao *M. haematochilus* were included by the preceding except the mouth which was 2.7.

It should moreover, be specially pointed out that the above mentioned points were located with the greatest care to secure uniformity. It was often difficult to decide just which scale row was below the origin or *immediately* behind posterior edge of fin. The minute scales were disregarded. In one specimen the rows of one side did not correspond with the rows of the other side but alternated (were staggered). Furthermore it will be seen that the count for both sides varies in four cases out of five. In one case there were five scales in the lateral line of the third area but six further up, in another there were ten scales on lateral line of first area but nine on the row just above it (it was counted as nine, plus an accessory). Thus it became obvious that these transverse rows were related to the position of the fins only in a very general way.

However, for comparison with *M. haematochilus*, an average was

worked out using the ten Tsingtao specimens. This is presented in figure 1 by uncovered numerals, while the corresponding figures for *M. haematochilus* are presented by numerals in parentheses. As the *M. haematochilus* counts are based on the two Tsingtao specimens, one of which was very irregular and the Asamushi specimens which were counted on a different basis, they should bear modification. The number of scales on "lateral line flanking the hypural plate, do *not* include the slender scale figured as 42 in my 1920 paper, and which I usually counted at that time.
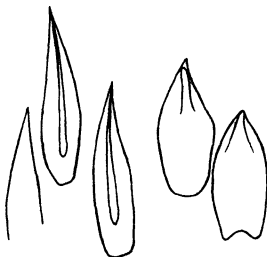
Fig. 2. Ctenu of scales of *M. cephalus* at *left*, *M. haematochilus* at right.

Scales (figure 2)

Teeth (those of periphery)

slender, 4.5 times as long as broad, sharply pointed, curved	a little over twice as long as broad, soon becoming dull-pointed, straight
with long, clearly defined central ridge	central ridge present only near apex
separated by more than width of central ridge	almost to quite touching
peripheral separated by flat interspace	no flat interspace

Number of radii varies with age and position on body.

SYSTEMATIC CONCLUSIONS.

From the data on number of vertebrae and number of transverse scale rows, it is evident that there is no, at least direct, corelation between the two. Both species have twenty-three vertebrae (plus the hypural). In both, the radialia (pterygiophore) of the first dorsal fin originate behind the spine of the sixth vertebra, of the second dorsal fin behind that of the thirteenth. Thus the skeletal characters of both are very similar. Yet the fins differ in the two species in relative position and the scale rows in numbers and disposition. Thus number of vertebrae, number and disposition of transverse scale rows, and relative position of insertion of fins are specifically constant but independent of each other — and may be independent specific (mutational) characters. The Asamushi M. cephalus had the first radialia of the dorsal fin inserted behind the spine of the seventh vertebra yet the first dorsal fins of these two equally large specimens of the two species were opposite, while the second dorsals, which were inserted behind the same vertebral spine (thirteenth) in both, was inserted 20 mm. in M. haematochilus behind that of M. cephalus (and the anal 25 mm.). Thus although the first dorsal originated further back osteologically, in M. cephalus, the ratios of the fin interspace was normal.

From the above data it will be seen that the two species are closely related differing chiefly in habits, development of adipose eyelid, number of anal fin rays, distance between dorsals, and relative size of scales — for, although M. haematochilus has two longitudinal rows less than M. cephalus, in specimens of the same size the difference is seen to be made up in size of scale. They appear very similar in the color pattern, number of vertebrae, point of insertion on vertebrae of second dorsal and anal fins, and the small difference in scale count (compared with other species). Thus we are confronted with two closely allied species, the range of the one being included in that of the other. In fact all species of mullet are included in the range of the one species M. cephalus. It is the writer's opinion that M. cephalus and M. haematochilus are very closely related phylogenically, having been separated in man's mind by undue emphasis on one structure which developes during the latest stage in development, i. e. the juvenile stage! Furthermore, judging from the variability of the material at

hand, the writer believes that the sperms of the one occasionally fertilize the eggs of the other. For instance the 565 mm. *M. haematochilus* had the following characters of *M. cephalus* though otherwise typical: slender build and slender peduncle (smaller than base of second dorsal), rows of scales above lateral line on left side and partly on right side, likewise an extra row below lateral line and extra rows on belly region, the scales being smaller, as if a regular row had split to form a double row, for longer or shorter distances, differing on the two sides of belly.

GROWTH AND MIGRATION

In this study it must be borne in mind that the same methods of study are used as outlined on pages 210-211 (1). For exact comparative work, scales must be taken from exactly the same spot on the fish, as the number of circuli vary in scales taken from different places.

The 419 mm. specimen of *M. cephalus* taken July 11th had a lateral circuli formula of 1.? : 44 + 24 which is very similar to Carolina mullets (p. 221) taken in October. This might indicate that the gonads developed earlier in the Japanese specimens.

The 241 mm. specimen of *M. haematochilus* taken the same day had a formula of 1.? + 20 + 20 + 20 (average of four scales). A glance shows that this corresponds to that of *M. cephalus* if the first two twenties are added, giving 1.? + 40 + 20. In other words *M. haematochilus* has a definite, rapid fall migration (contrasting with the leisurely fall migration of *M. cephalus* which does not interrupt growth) which so interrupts growth as to break the circuli; and having four circuli less than *M. cephalus* either adds them more slowly (though the scale is larger) or migrates a month later, i. e. early May (if growth of scale and fish are approximately equal). The two Tsingtao specimens had an average formula of 1.? + 26 + 12 + 10 (555 mm.) and 1.? + 15 + 14 + 11 (565 mm.). Thus again one is confronted with the phenomenon of two migrations per annum thought not at such regular intervals as the Asamushi specimen.

The four young taken on the 11th of July had twelve to eighteen (average fourteen) postlinea circuli. If the rate of accretion of circuli

is equal to that of *M. cephalus* (i. e. about five per month) this would indicate that they had grown three and a half months since migration or had migrated in mid-April. As the record of the single adult indicates an early May migration, one may hold, as a working hypothesis, that *M. haematochilus* migrates to northern Japan near the end of April.

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